SPATIAL AND TEMPORAL ANALYSIS OF SARS-CORONAVIRUS-2 CONCENTRATION IN WASTEWATER AND ITS ASSOCIATION WITH OTHER EPIDEMIOLOGICAL INDICATORS IN THE NETHERLANDS

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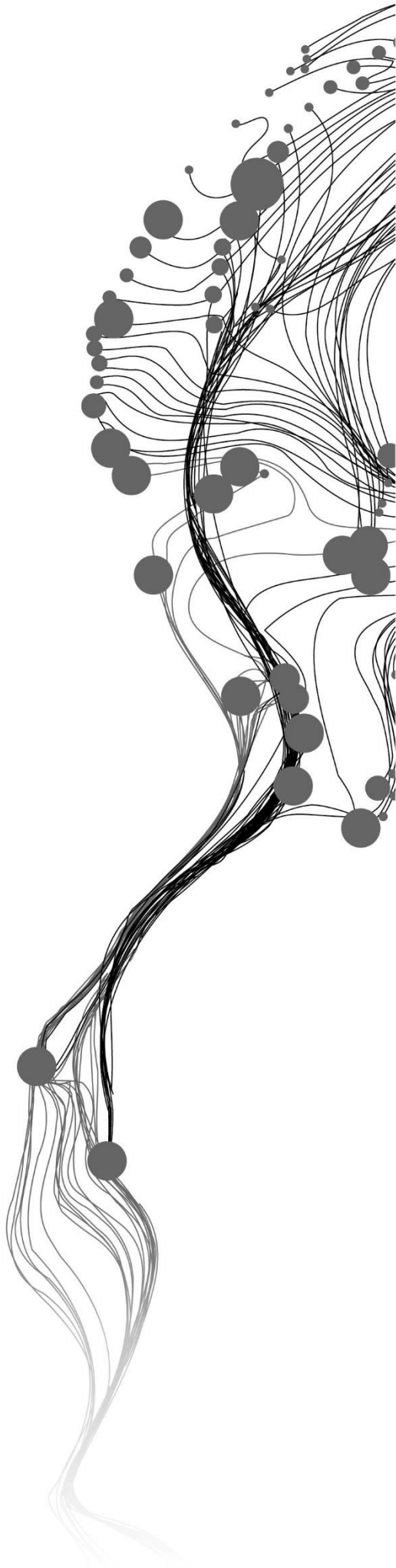
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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a public health emergency of global concern since its outbreak in 2019/2020. In order to monitor the spatial and temporal patterns of SARS-CoV-2 transmission, public health authorities and universities have established a variety of dashboards worldwide. These dashboards usually track multiple epidemiological indicators such as the number of confirmed COVID-19 cases, hospital admissions, deaths. In the Netherlands, wastewater surveillance of SARS-CoV-2 is used as additional indicator serving as an early warning for (re-)emergence of SARS-CoV-2 circulation in communities. Our research assesses the added value of the SARS-CoV-2 wastewater RNA concentration data in relation to the standard epidemiological indicator data collected in the Netherlands across space and time.

Publicly available secondary data on SARS-CoV-2 wastewater concentration and the epidemiological indicators were compiled into a unified spatial database involving data fusion and spatial (dis-)aggregation. Subsequent analysis consisted of three stages. In stage 1, the spatio-temporal patterns of SARS-CoV-2 transmission were visualised through time series mapping at multiple geographic scales. In stage 2, the statistical association between all epidemiological indicators was assessed based on bivariate correlation analysis. In stage 3, the feasibility of using SARS-CoV-2 wastewater data – as a spatially disaggregate early warning indicator of transmission intensity – was determined based on cross-correlation analysis.

Spatio-temporal patterns of SARS-CoV-2 wastewater RNA concentration data and epidemiological indicators across geographical scales (national, safety region and municipality level) can be identified. Overall, SARS-CoV-2 concentrations in wastewater have a medium to strong correlation with the weekly number of confirmed COVID-19 cases. Considerable variations, however, are apparent across municipalities and the (much larger) safety regions. Cross-correlation results indicate that SARS-CoV-2 wastewater concentrations precede confirmed positive cases in most municipalities in the Netherlands. This indicates that SARS-CoV-2 wastewater concentration data has potential as an early warning indicator useful for the public health officials to make informed decisions.

Our novel spatio-temporal approach shows that SARS-CoV-2 wastewater concentration data has added value as a complementary epidemiological indicator. We recommend further testing of our methodology.

Keywords: COVID-19, disease surveillance, SARS-CoV-2, space-time patterns, wastewater-based epidemiology.

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LIST OF ACRONYMS AND GLOSSARY

Abbreviations

Abbreviations	Details
ARIMA	Auto-Regressive Integrated Moving Average
CCF	Cross Correlation Function
ICU	Intensive Care Unit
MERS	Middle East Respiratory Syndrome
PM	Particulate Matter
RIVM	The National Institute for Public Health and the Environment
SARS-CoV-2	Severe Acute Respiratory
WBE	Wastewater-Based Epidemiology
WWTP	Wastewater Treatment Plants

Glossary

The research glossary describes the terms used in reporting of the coronavirus in this research.

Terminology	Definition
Acute respiratory distress syndrome (ARDS)	A health condition where the lungs is unable to provide oxygen to the vital organ.
Aerosol	Suspended tiny particles in the air. When an infected person exhales, the virus droplets is let out and suspended in the air, thereby generating aerosol.
Antibody response	An antibody is produced by the body's immune system in reaction to a virus infection. Antibody response involves the presence of antibodies in the blood. The presence of the coronavirus antibodies in the blood indicates that the person has been infected with the virus at some point. However, the person might not show the symptoms when infected.
Asymptomatic	An infected person who does not show symptoms of the coronavirus. Despite not showing symptoms of the coronavirus, it is possible an asymptomatic person can spread the virus to others.
Animal to human transmission	This involves the transmission of an infection from an animal to human through contact and the presence of the virus in the air.
Biomarkers	These are characteristics, structures, or processes that can be measured in the wastewater to describe the presence or occurrence of drugs or viruses.
Blood transmission	The transmission of the SARS-CoV-2 virus through transfusion of blood.
Clinical-based surveillance	The systematic collection, analysis, and interpretation of health-related data to enable public health practitioners to make informed decisions.
Confirmed cases	The number of people confirmed to be positive for the SARS-CoV-2 virus
COVID-19	Acronym for coronavirus disease in 2019. COVID-19 is the name of the disease

	caused by the highly infectious coronavirus (SARS-CoV-2) after its outbreak in late 2019.
Diagnose	An identification of the nature of a medical condition as a result of an examination of symptoms
Diarrhoea	Loose or watery stools, a characteristic of a gastrointestinal disorder
Droplet	A tiny particle exhaled when an infected person talks, sings, coughs, or sneezes. One can contract the virus when in closed contact with the droplet from an infected person without precautions like physical distancing, adequately ventilated environment, and the use of face masks.
Endogenous	A growth from an internal cause
Epidemic	A rapid and unexpected increase in the number of infections of a disease in a population.
Epidemiological indicators	This includes the number of hospital admissions, positively tested cases, mortality, Intensive Care Unit (ICU) admissions, R number, and nursing homes confirmed cases for monitoring the spread of COVID-19.
Exogenous	A growth from an external cause
Faecal-oral transmission	Route of disease transmission from person to person, where fecal matter is consumed orally. Disease transmission through consumption of faeces of an infected person by another person.
Fomite	An object which carries infectious agents when contaminated, therefore passively enabling transmission to a new host.
Gastrointestinal symptoms	It involves symptoms of SARS-CoV-2 virus such as vomiting, diarrhea, stomach pain and constipation.
Gross Domestic Products (GDP)	The monetary value of the goods and services produced by a country's economy in a specific time period.
Incubation period	The period between infection and the appearance of first signs.
Infectivity	The ability of pathogens of disease to infect many people.
Infectivity rate	The rate of the spread of a disease in a population
Intensive Care Unit	It is a section of the hospital where critically ill patients are looked after.
Malaise	A state of uneasiness or discomfort, usually the first sign of an infection.
Mammals	Warm-blooded animals with back bones.
Myalgia	Medical term for muscle pain caused by injury, infections, or inflammatory condition.
Middle East Respiratory Syndrome	It is a viral disease of the coronavirus family spread between animals and humans. The virus can be contracted through physical direct and indirect contact with infected people or animals.
Morbidity	The incidence rate of a disease in a population.
Mortality	The number of deaths in a population in a certain period.
Mutation of SARS-CoV-2 virus	The change in form of the structure of the SAR-CoV-2 virus in the creation of a variant form.
RIVM	The National Institute for Public Health and the Environment is an independent research institute in the Netherlands conducting research for public health safety and a good living environment.

Pandemic	Epidemic of an infectious disease over a wide geographical area and affecting a large population.
Pneumonia	Inflammation of the lungs resulting from a viral or bacterial infection.
pH	A measure of acidity or basicity of solutions.
Physical distancing	Staying about 1.5 meters away from other people to prevent the spread of the virus.
Presymptomatic infections	The early stages of a disease infection before all symptoms develop
Positive tested cases	The patients that tested positive for the coronavirus.
Psychoactive substances	Drugs and other substances that affect one's brain or mental health when consumed.
R – number	This is described as the reproduction number, which indicates the speed of the spread of the virus. It describes the average number of people infected on average by an infected person.
Serologic testing	A test to determine the presence of components of serum such as antibodies and antigens in a patient's sample.
SARS-CoV	Acronym for severe acute respiratory syndrome coronavirus disease is a viral disease belong to the coronavirus family, which affects the respiratory system of infected people. The disease shares the same characteristics as the other coronavirus diseases.
SARS-CoV-2	Acronym for Severe Acute Respiratory Syndrome coronavirus 2 also belongs to the coronavirus family and affects the human respiratory system. The SARS-CoV-2 is the cause of the global pandemic, COVID-19.
Social distancing	It is a measure that involves the people staying at home to curb the spread of a virus.
Symptomatic	Exhibiting signs of infection or symptoms of a disease.
Wastewater-based epidemiology	An approach that provides information about exposure to a disease in a population through the analysis of human excretion.
Wastewater treatment plants	A wastewater treatment plant removes impurities from sewage so that they can return to the environment such as aquifers or natural water bodies.

1. INTRODUCTION

1.1. Background and Justification for the study

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a public health emergency of international concern since its outbreak and its declaration as a pandemic in March 2020 by the Director-General of the World Health Organisation (WHO) (Sohrabi et al., 2020; WHO, 2020g; Zhou et al., 2020a). COVID-19 is a novel virus which belongs to the family of Coronaviridae that infects humans and mostly affects the respiratory system (Hu et al., 2021). In the past, outbreaks of the family Coronaviridae caused deadly respiratory sickness in humans, including the Middle East Respiratory Syndrome (MERS-CoV-2) in 2002 and the Severe Acute Respiratory Syndrome (SARS-CoV) in 2012 respectively; thereby making developing coronaviruses a new public health concern (Cui et al., 2019a). While there are several coronaviruses, the SARS-CoV-2 virus is one of the most fatal in the coronavirus family, causing slight to severe respiratory illnesses and death. In 2019, the SARS-CoV-2 virus emerged from Wuhan, China (Huang et al., 2020) and has since spread to over 200 countries and territories across the globe with over 205 million infected people and 4.3 million reported deaths, unusual of any viral pneumonia outbreak in the 21st century (WHO, 2021d). The SARS-CoV-2 has a high infectivity rate causing the spread of the virus within a short period (Remuzzi & Remuzzi, 2020). With the spread of the virus to different countries and territories all over the world, different mutations of the SARS-CoV-2 virus have emerged; thereby producing several variants of the SARS-CoV-2 virus (Conti et al., 2021). The alarming rate of transmission of SARS-CoV-2 has led to a disruption of regular way of life and of healthcare systems around the globe; it is an unprecedented crisis that affects all dimensions of life, movement, and economic activities.

The outbreak of the SARS-CoV-2 virus has led to global and local measures from countries to tackle this burning public health concern (Rahimi et al., 2020). Measures such as social distancing, physical distancing, quarantine, promotion of personal hygiene through handwashing and use of hand sanitizer, and the use of a facemask are being implemented to curb the spread of the virus. These measures have shown to be effective in minimising the number of COVID-19 infections (Bragazzi et.al, 2021; Chiu et al., 2020). Also, improvement in the supply of personal protective equipment (PPE), medical devices such as respirators and oxygen, and development of a vaccine are effective measures for the treatment of infected persons and the protection of people against the virus respectively (Flaxman et al., 2020; Han et al., 2020).

Other measures such as international flight restrictions and local travel restrictions have proven to reduce the spread of the virus (Chinazzi et al., 2020). These measures are challenging to implement; and have positive and negative impacts. For example, flight restrictions between nations aimed at curtailing the virus led to disruptions of travel, businesses, human interaction, social gatherings (Guan et al., 2020). Kumar et al. (2020), identified some positive environmental impact of the measures such as decreased air and noise pollution, reduced anthropogenic pollution of beaches, reclamation of territories by wildlife. Also, across countries, restriction of movement and other lockdown measures helped decrease carbon footprint (Anil et al., 2020; Wang et al., 2020). Some negative effects of these measures include increased organic and inorganic waste generation through disposal of personal protective equipment (PPE), increase in food wastes and household wastes causing environmental pollution (Yudell et. al., 2020; Zambrano-Monserrate et.al., 2020). Measures, such as quarantine, social and physical distancing, face covering have also changed human (social and physical) interactions; with implications for mental health.

Further actions and regulations on travel restrictions, social and physical distancing to curb the spread of the SARS-CoV-2 virus have taken a significant toll on the economy, employment rate, and trade activities across the globe (Gössling et al., 2020). From the beginning of the pandemic in 2019 till 2020, the world experienced an economic decline, with reports from the World Bank, (2020) indicating a 5.2% global economic shrinkage, and the European Central Bank estimated a 12% contraction for the Eurozone economy in 2020 (Eurostat, 2020). The Gross Domestic Product (GDP) used to determine the wellness of a nation's economy fell by 32% in the United States of America, by 27.8% in Japan (Dennis, 2020), by 20.4% in the United Kingdom, and by 8.5% in the Netherlands (CBS, 2020a) by the second quarter of 2020. The United Nations (2020), estimated a decline in global trade by approximately 13% to 32% due to the worldwide measure and restrictions. Recently, the Bureau of Statistics Netherlands, (2021) reported a further contraction of GDP in the Netherlands by 0.5% in the first quarter (January – April) of 2021 relative to the third quarter (September – December) of 2020. There is a large geographical variation not only in implementation but also in the effects of these actions and regulations to stop the spread of the virus (Verschuur et. al., 2021). With continuous spread or resurgence of the SARS-CoV-2 virus, the economy will face further challenges and would lead to more global economic hardship.

Considering the high infectivity of the SARS-CoV-2 virus and the adverse social and economic effects of the measures to contain it, it is necessary to understand its transmission and spread for proper surveillance and monitoring (Ferretti et al., 2020). The surveillance of the SARS-CoV-2 virus through monitoring of the trends in the epidemiological indicators such as hospital admissions, mortality, and positive tested cases from clinical tests supports public health authorities (WHO, 2020c). The rate of clinical testing for the virus has increased over time with more production of test kits and expansion and distribution to meet with the demands. Still, testing all the population would be costly and require a lot of workforce. For example, in some countries, people are tested based on set rules and requirements, such as testing symptomatic patients and those who had travelled to high-risk regions or countries according to CDC (2020) in the United States of America (U.S), and the Netherlands (RIVM, 2021). Yuan et al. (2021) discussed that the people infected with the SARS-CoV-2 virus experience stigmatisation, thereby, affecting their psychological and mental health. Hence, clinical testing might result in people being afraid of stigmatisation associated with positively confirmed cases of the disease. However, with the inadequacies in clinical testing, both symptomatic, asymptomatic and pre-symptomatic patients might not be correctly accounted for in the estimation of SARS-CoV-2 confirmed cases for decisions on public health (Murakami et al., 2020). Serologic testing which involves measuring the antibody response of individuals for infection of SARS-CoV-2 has shown a premise for identifying the symptomatic and asymptomatic infection rates; and the trend of the virus within a specific population (Yongchen et al., 2020). Serology would allow understanding of the number of severe cases among infected people, but testing a whole population is costly and requires lots of people. Therefore, a cost-effective technique that requires limited workforce for real-time surveillance of the SARS-CoV-2 virus that can improve public health decisions is needed.

Wastewater-based epidemiology (WBE) is an approach used to measure chemical and biological pollutants in wastewater, based on the quantitative and qualitative analysis of the data obtained from the wastewater generated by the population within the wastewater treatment plant (Choi et al., 2018). WBE can be used to explain the trends of events in a population through analysis of the biomarkers in the wastewater within the population. It offers another approach to describe the spatial and temporal trends of illicit drug consumption when interpreted with other indicators such as drug-use surveys and drug impounded (Kankaanpää et al., 2016). Studies on WBE have focused on illicit drug and alcohol consumption (Lorenzo & Picó, 2019; Mercan et al., 2019; van Nuijs et al., 2011; González-Mariño et al., 2020; López-García et al., 2019), but also offer premise in the monitoring of the spread of diseases within a population.

Some studies describes the vital role that WBE played in the early warning and supervision of enteric viruses like poliovirus, norovirus (Flannery et al., 2012; Nordgren et al., 2009), and hepatitis virus (Cuevas-Ferrando et al., 2020; Miura et al., 2016). WBE has shown potential in signalling the spread of these viruses within a population not captured by clinical testing through analysis of both symptomatic and asymptomatic people. The prospects of WBE have developed rapidly and shown to provide critical and extensive early surveillance analysis of public and environmental health statuses at the community level (Mao et al., 2020).

Researchers have been considering wastewater testing as a feasible means of assessing and monitoring the SARS-CoV-2 virus across countries to complement results derived from clinical and serologic tests of the virus. Diarrhoea has been reported as a possible symptom of COVID-19, with both symptomatic and asymptomatic individuals shedding the SARS-CoV-2 RNA (ribonucleic acid) through faeces (Han et al., 2020; Lo et al., 2020). The existence of SARS-CoV-2 RNA in faeces brings about research on coronavirus in wastewater (Wang et al., 2005). Wu et al., (2020) explain that the presence of SARS-CoV-2 RNA in faeces or stools of infected people weeks after the infection is not detected with clinical oral tests. With WBE as a possible support tool for the surveillance of the SARS-CoV-2 virus, studies have shown the presence of the virus in wastewater in different countries (Ahmed et al., 2020a; Kumar et al., 2020; Sherchan et al., 2020), including the Netherlands (Medema et. al., 2020). The presence and detection of the SARS-CoV-2 RNA in wastewater provides an opportunity for another approach to monitor and describe the spatial and temporal spread of the virus within a population.

 In The Netherlands, the National Institute for Health and Environment (RIVM) monitors the spread of COVID-19 using different epidemiological indicators through the coronavirus dashboard (RIVM, 2020b). These indicators describe the intensity of the infection of SARS-CoV-2 virus at different temporal and spatial units. With the weekly measurement of the SARS-CoV-2 concentration from wastewater, the number of coronavirus particles in wastewater treatment plants (WWTP) has been included on the dashboard. Also, the datasets of these epidemiological indicators, including the SARS-CoV-2 wastewater concentration, exists on different open data sources published by the RIVM. Therefore, the identification, integration, and organisation of the data sources to provide an improved understanding of the spatial and temporal analysis of the SARS-CoV-2 virus is worth exploring. The combination of WBE with other data sources of epidemiological indicators would provide a comprehensive spatial and temporal analysis of health-related issues. Therefore, integrating WBE with other data sources of epidemiological indicators would provide a comprehensive explanation of the potentials of epidemiological indicators in observing the spatial and temporal trends of SARS-CoV-2 virus (Mao et al., 2020).

Studies on the SARS-CoV-2 concentration in wastewater have also shown the association between wastewater samples and confirmed cases in a population (Ahmed et al., 2020; La Rosa et al., 2020; Rimoldi et al., 2020), but none of these studies assessed the potential of the data in describing the spatial and temporal trend of the virus. In the Netherlands, Medema et al. (Medema et al., 2020) investigated the detection of SARS-CoV-2 concentration in wastewater and described the potential of the WBE to measure the trend of infection rates at the early stages of the pandemic. Their research addressed the relationship between the concentration of SARS-CoV-2 RNA in wastewater, and the confirmed cases estimated and suggested the comparison of positive cases with the SARS-CoV-2 concentration in wastewater based on the catchment area of the WWTP. The study also described that the temporal pattern of the SARS-CoV-2 concentration in the WWTP of eight municipalities corresponds with confirmed positive cases of the virus. Medema et al. (2020) suggested the need for accurate quantification of SARS-CoV-2 virus RNA in wastewater and the availability of more epidemiological data at different scales to demonstrate the relationship between the SARS-CoV-2 virus RNA in wastewater and the other

epidemiological indicators. There has been a continuous incorporation of the wastewater concentration data for the WWTP to the dashboard managed by RIVM. Also, there has been an update of the other epidemiological indicators characterised by varying spatial units on the dashboard. With the update and incorporation of these dataset on the coronavirus dashboard, it is therefore essential to assess the prospect of SARS-CoV-2 concentration in wastewater in analysing the spatial and temporal patterns of the virus through comparison of the relationship between the epidemiological indicators.

Research on the detection of SARS-CoV-2 virus in wastewater have explored the relationship between the wastewater RNA and some other epidemiological indicators and proposed that other methods can be further explored to describe the relationship between the SARS-CoV-2 in wastewater and the other epidemiological indicators (D'Aoust et al., 2021; Nemudryi et al., 2020). Employing suitable methods in analysing the association of the epidemiological indicators of SARS-CoV-2 virus is vital in understanding the virus (Farkas et. al., 2020). Studies related to COVID-19 and SARS-CoV-2 virus have been exploring the geospatial and geostatistical techniques in monitoring and surveillance of the virus (Franch-Pardo et al., 2020; Boulos et al., 2020). The research reviewed studies that involve the application of GIS techniques and methods to different aspects of COVID-19, including spatiotemporal analysis, disease mapping, and environmental impact of the virus. These studies propose the use of GIS techniques along with geostatistical techniques to proffer solutions to new issues related to coronavirus. Also, Franch-Pardo et al. (2020), describe that the use of geospatial methods would provide more understanding of the spatial and temporal dynamics of the SARS-CoV-2 virus, which is important for its mitigation. They also explained that a comprehensive spatial and temporal analysis of the virus would give more insight into the impact of the pandemic, hence, helping decision making, planning and mitigation measures. The application of statistical and geospatial techniques has shown potential in providing evidence for mitigation measures and decision making to curb the spread of the virus.

Researchers advised that the feasibility of WBE as a public health tool for monitoring the SARS-CoV-2 concentration in wastewater should not take away the clinical testing of individuals but rather complement the epidemiological indicators (Farkas et al., 2020; Xagoraraki & O'Brien, 2020). They suggested the integration of WBE with other public health indicators to assess the prevalence of the virus in a population to proffer mitigation measures. There is limited research that explains the relationship between the SARS-CoV-2 concentration in wastewater and the other epidemiological indicators across the national scale —for example, understanding the spatial and temporal relationship between hospital admissions and SARS-CoV-2 concentration in wastewater, positive confirmed cases, and SARS-CoV-2 concentration in wastewater, and coronavirus deaths and SARS-CoV-2 concentration in wastewater. There is a need to understand the significance of these epidemiological indicators to assess the potential of the SARS-CoV-2 concentration in wastewater. Hence, identifying these epidemiological indicators from the different sources and organising suitably, to identify their relationship with SARS-CoV-2 concentration in wastewater is worth exploring. These would help to describe the potential of SARS-CoV-2 concentration in wastewater as a feasible way to measure the virus spread. Also, the result of this analysis would support informed decision-making and, eventually, policy development.

1.2. Problem Statement

COVID -19 is a complex and highly infectious novel disease, which underlies strong spatio-temporal dynamics. Understanding the spatial and temporal trends through different means of surveillance is challenging. Standard approaches using the number of positively tested cases (daily/weekly), the percentage of positively tested cases, R number, hospital and ICU admissions data, positive cases in nursing homes data, mortality and morbidity rates obtained from clinical-based surveillance, serological testing, and surveys are different ways of monitoring the COVID-19 transmission. These methods

represent a measure of active cases while testing the entire population is impractical and expensive for most countries. The detection of the SAR-CoV-2 RNA in faeces and the quantification of the virus particles from stools and wastewater holds potential for another approach to understand the spatial and temporal trends of the virus as proven with other viral diseases, although the potential of the SARS-CoV-2 concentration in wastewater has not been fully explored. No single data source can fully provide a comprehensive understanding of the spatial and temporal trends of the disease. Therefore, a multi-indicator approach is needed.

Building on the work of Medema et al. (2020)'s work on relationship between SARS-CoV-2 wastewater RNA and reported COVID-19 cases, this research explores the spatial and temporal trends of selected epidemiological indicators. These epidemiological indicators exist in different data sources, which are unique and consist of the underlying patterns over time. The potential of the SARS-CoV-2 wastewater data as an early warning indicator to monitor the severity of the virus in the Netherlands is explored. The resulting temporal analysis would be compared across space and see where they differ and complement each other. The comparison of the spatial and temporal analysis of the different data sources would help clarify the relationship between the data sources and support informed decision making. Also, identifying the relationship between the SAR-CoV-2 concentration in wastewater and other epidemiological indicators to examine the potential of WBE in monitoring the spatial and temporal trends of SAR-CoV-2 is yet to be studied.

Therefore, this research aims to compare the spatial and temporal patterns of SARS-CoV-2 wastewater data and selected epidemiological indicators as a feasible way to describe the spatial and temporal variation of the SARS-CoV-2 virus in the Netherlands.

1.3. Research Objectives and Questions

1.3.1. Research Objectives

The main research objective is to develop a comparative approach to assess the potential of SARS-CoV-2 wastewater data in relation to other epidemiological indicators and analyse the spatial and temporal trends of SARS-CoV-2 virus in the Netherlands. The research objective is further divided into three sub-objectives identified as follows:

1. To establish reliable data sources of epidemiological indicators suitable for spatial and temporal analysis of SARS-CoV-2 in the Netherlands.

2. To generate spatial and temporal patterns for the epidemiological indicators and SARS-CoV-2 wastewater data, and identify the similarities and differences based on the patterns.

3. To derive evidence-based recommendations for health-related decision making and policy development based on the similarities and differences of the spatial and temporal patterns of epidemiological indicator data and SARS-CoV-2 wastewater data.

1.3.2. Research Questions

1. **To establish reliable data sources of epidemiological indicators suitable for spatial and temporal analysis of SARS-CoV-2 virus in the Netherlands.**

    a) Which data sources of epidemiological indicators exist for spatial and temporal analysis of the SARS-CoV-2 virus?

b) What are factors that determine the epidemiological indicators of SARS-CoV-2 virus?

c) How can the suitable epidemiological indicators be organised for spatial and temporal analysis?

2. **To generate spatial and temporal patterns for the epidemiological indicators and SARS-CoV-2 wastewater data, and identify the similarities and differences based on the patterns.**

a) What methods are best suited to describe the spatial and temporal patterns of epidemiological indicators and SARS-CoV-2 wastewater data in wastewater-based epidemiological studies?

b) What are the similarities and differences between the spatial and temporal patterns of the epidemiological indicators and SARS-CoV-2 wastewater data?

c) Can SARS-CoV-2 wastewater data be used as an (early warning) indicator of virus transmission intensity?

3. **To derive evidence-based recommendations for health-related decision making and policy development, based on the similarities and differences of the spatial and temporal patterns of epidemiological indicator data and SARS-CoV-2 wastewater data.**

a) How can the resulting similarities and differences between the spatial and temporal patterns of the epidemiological indicators and SARS-CoV-2 wastewater data be used for decision making and policy planning?

1.4. Thesis Outline

Chapter 1 provides a general overview and background information on the epidemiology of COVID-19 and the causative virus, SARS-CoV-2. It highlights the transmission media, symptoms, impacts, actions, and regulations of the SARS-CoV-2 virus. It also discusses the approach of WBE as a possible means to understand the spatial and temporal trend of SARS-CoV-2 virus. It identifies the research problem statement, the research objectives and highlights the research questions to achieve the objectives.

Chapter 2 presents the findings of a review of key literatures on the epidemiology of COVID-19 and the approaches for surveillance of the SARS-CoV-2 virus. It describes the existing knowledge on WBE and its application to viruses, drugs, and alcohol consumption and provides focused examples of studies on these topics. The chapter also gives an understanding of the relation between the WBE approach and the standard epidemiological indicators and their impact on each other. This chapter highlights the methods employed in past literatures to establish relationship between the concepts on similar topics.

Chapter 3 defines the methodology employed at different stages of the research and the ethical consideration. It also provides an overview of the research methodological workflow, describing the case study selection, the methods for data acquisition, processing and organisation, the selection of suitable methods, implementation of the methods suitable for spatial and temporal techniques, and finally, the comparative analysis of the spatial and temporal analysis of the SARS-CoV-2 wastewater and the epidemiological indicators.

Chapter 4 presents the results of the spatial and temporal trends and patterns of the SARS-COV-2 concentration in wastewater data and the selected epidemiological indicators. It presents the result of the statistical association between all the epidemiological indicators and describes the spatial variation of the statistical relationship across different geographic scales. It outlines the output of the temporal relationship between SARS-COV-2 concentration in wastewater and the selected epidemiological indicators and presents the spatial patterns across different geographic scales.

Chapter 5 discusses the result of the thesis. First, the discussion on the temporal exploration of all the epidemiological indicators, followed by a discussion of the spatial patterns of SARS-COV-2 concentration in wastewater data and the selected epidemiological indicators during the study period. It discusses the results of the statistical and temporal association and between epidemiological indicators and their spatial patterns. Finally, the evidence-based recommendation is discussed based on the comparative analysis spatial and temporal analysis of the SARS-CoV-2 concentration in wastewater and the selected epidemiological indicators. It also highlights the limitation of the research.

Chapter 6 outlines the conclusions for the thesis and provides recommendations for future research.

2. LITERATURE REVIEW

2.1. Introduction

The coronavirus pandemic has been one of the most challenging global health threats in recent time (Park et al., 2021; WHO, 2021a). Due to the complexity of the virus, different measures need to be adopted to provide a comprehensive understanding of the spatial and temporal spread of the SARS-CoV-2 virus (Wu & Zhang, 2021). Different countries all over the world have adopted the standards means of clinical and serologic testing to monitor the spread of the virus, thereby providing mitigation measures at national and regional scales (Damluji et. al., 2021; La Marca et al., 2020; Mercer & Salit, 2021). At present, WBE is being adopted in different countries, including the Netherlands, as another approach to describe the spread of the virus within a population with consideration for the infected people, which are limited by clinical and serological testing. Therefore, this chapter introduces the epidemiology of COVID-19 and an overview of the transmission, symptoms, surveillance approaches of the SARS-CoV-2 virus. It describes the role of WBE as a measure of the spatial and temporal trends of viruses and other related studies. It also describes the methods adopted in different studies to describe the spatial and temporal spread of the SARS-CoV-2 virus.

2.2. Epidemiology of COVID-19

Corona means crown in Latin, was named from the crown-like spikes on the outer surface of the virus, Coronavirus (Rabi et al., 2020). Coronavirus (CoV) is a member of the virus family 'Coronaviridae' and the subfamily 'Coronaviridae' (ICTV, 2011). The CoVs have about four main groups, including alpha, beta, gamma, and delta. Mammals are infected with the alpha and beta coronaviruses causing cold, abdominal pain, diarrhea and respiratory diseases in humans and animals (Cui et al., 2019a; Zhou et al., 2018). The gamma and delta coronaviruses infect mammals and birds (Chen et al., 2020). Presently, seven different coronaviruses have been discovered to infect humans. In contrast, four human coronaviruses (HCoVs) such as H-CoV-OC43, HCoV-NL63, HKU1, HCoV-229E have been reported to cause common colds and infections in the elderly and infant population (Dhama et al., 2020). These four HCoVs causes mild to moderate diseases. The other three coronaviruses include the SARS-CoV, MERS-CoV and the new SARS-CoV-2 which causes mild to serious cold, pneumonia and respiratory illnesses in humans (Cui et al., 2019b). The SARS-CoV and the MERS-CoV had emerged earlier, resulting in high infections and mortality and was a public health concern. The summary of the characteristics of the SARS-CoV, MERS-CoV and the novel SARS-CoV-2 virus is as displayed below (Table 1). Based on the discovery of the SARS-CoV, some studies have investigated the epidemiological effects of the HCoVs, further leading to the different methods adopted in the identification of the virus, such as testing the secretion from human respiratory outlets (Bosis et al., 2007; Fouchier et al., 2004; Prompetchara et al., 2020). They also demonstrated that the SARS-CoV could be considered to have a zoonotic origin, considering its transmission from animals to humans (Guan et al., 2003), with the transmission of the SARS-CoV, MERS-CoV and SARS-CoV-2 to humans were reported to have occurred in an animal market (Guan et al., 2003; Hemida et al., 2013; Rabi et al., 2020).

Table 1: Characteristics of SARS-CoV, MERS-CoV and the SARS-CoV-2 viruses summarised by Ganesh et al. (2021)

Characteristics	SARS-CoV	MERS-CoV	SARS-CoV-2	References
1. Origin (Year)	2002	2012	2019	(Prompetchara et al., 2020)
2. Origin (Location)	Guangdong, China	Jeddah, Saudi Arabia	Wuhan, China	(Xie & Chen, 2020)
3. Confirmed cases	8,098	2,279	167,011,807 [nb]	(WHO, 2021b, 2021c)
4. Mortality	774	806	3,472,068 [nb]	(WHO, 2018, 2021b, 2021c)
5. Number of countries and territories involved	29	27	237 [nb]	(Z. Song et al., 2019)
6. Incubation period	2-10 days	2-14 days	1-14 days	(Lee & Hsueh, 2020)
7. Transmission means	Physical contact with an infected person, and through respiratory droplets of an infected person	Physical contact with an infected person, and through respiratory droplets of an infected person	Physical contact with an infected person and through respiratory droplets of an infected person	(Tu et al., 2020)
8. Human-to-human transmission	Effective	Reduced	Effective	(Lee & Hsueh, 2020)
9. Transmission coverage	Global coverage	Region	Global coverage	(Z. Song et al., 2019)
10. Pandemic possibility	Yes	No	Yes	(Lee & Hsueh, 2020)
10. Symptoms	Headache, cough, diarrhea, fever, myalgia, shortness of breath, and malaise	Diarrhea, fever, myalgia, shortness of breath and cough	Cough, fever, shortness of breath, diarrhea, sore throat, and myalgia	(C. C. Lai, Shih, Ko, Tang, & Hsueh, 2020; Tu et al., 2020)
11. Complications	Severe acute respiratory distress syndrome, pneumonia, death	Severe acute respiratory distress syndrome, pneumonia, death	Severe acute respiratory distress syndrome, pneumonia, death	(C. C. Lai, Shih, et al., 2020)
[nb] : Information about the SARS-CoV-2 extracted from WHO (2021c) as of 25th May 2021.				

The outbreak of the SARS-CoV-2 virus was first discovered in Wuhan, China, in December 2019 with early identification of clusters of pneumonia by the Wuhan health authorities, and several measures for different classes of infectious diseases were adopted to curb the spread of the virus (Hasnain et. al., 2020). An independent panel of experts established by the WHO believes stricter measures could have been imposed earlier by the Chinese authorities and WHO could have acted faster to prevent the subsequent global spread of the virus (The Independent Panel for Pandemic Preparedness and Response, 2021). The report explained that the level of response to contain the virus was insufficient to meet the high transmission rate of the virus. According to the same report, following the declaration of the SARS-CoV-2 virus as a public health emergency of international concern on 30 January 2020, countries around the

world were reluctant with their approach on control strategies to curb the spread of the virus. Also, the declaration of the lockdown in Wuhan, China on 23rd February 2020, brings more scare on the severity of the SARS-CoV-2 virus that causes severe public health concerns (Zheng, 2020). Subsequently, the virus spread continuously to different countries and regions, with over 82,294 reported cases and 2,804 deaths in 47 countries as of 27th February 2020 (WHO, 2020b). Zheng (2020) summarised some of the main events in the early stages of the COVID-19 (Figure 2-1)
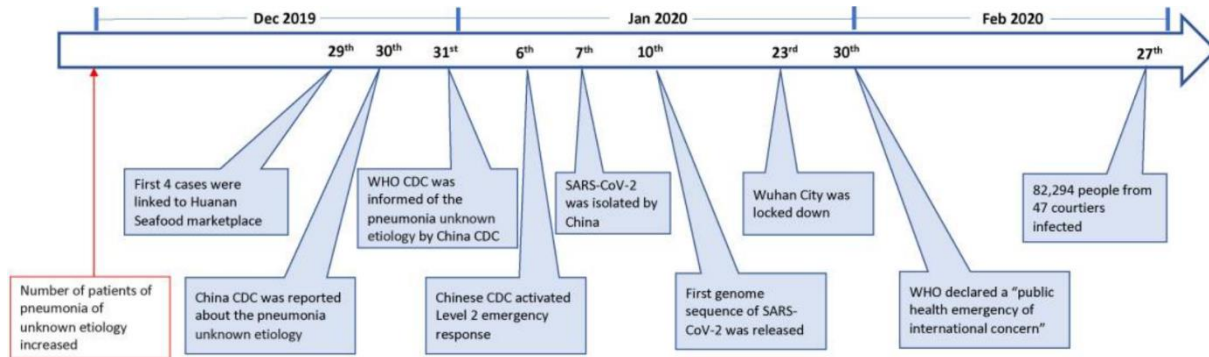
Figure 2-1: Some main events in the early stages of the outbreak of the SARS-CoV-2 adapted from Zheng, (2020)

The SARS-CoV-2 has proven to be more infectious than the SARS-CoV and MERS-CoV viruses due to its high transmission rate and difficulty locating infected people with mild symptoms and presymptomatic people (Ferretti et al., 2020). Understanding the transmission of the virus would give more insight into the spread of the SARS-CoV-2; hence, reducing the infection rate and its associated deaths (Chowdhury & Oommen, 2020). The early infected persons were reported to have contact with the wet market, which explains the possible early transmission of the virus from animals to humans, which has now evolved into human-to-human transmission (Zhou et al., 2020b). But there is still an ongoing investigation by the WHO to establish the source of transmission of the SARS-CoV-2 during the early stages causing the outbreak of the virus. The WHO explained that the transmission of SARS-CoV-2 is possible through multiple transmission pathways such as contact, airborne, droplet, fomite, faecal-oral, mother-to-child, animal to human transmission, and blood transmission, as shown in Figure 1 (WHO, 2020f). The SARS-CoV-2 can be transmitted through direct physical contact, or indirect physical contact with an infected person, contaminated environment, or contaminated surfaces or objects (fomites) through means such as droplets in the breath, saliva, and respiratory droplets (Karia et al., 2020). The transmission is mainly from contact with an infected person, when the infected person talks, sings, coughs, or sneezes without adequately covering their mouth and nose and exhales infected secretion such as respiratory droplet and saliva (Ghinai et al., 2020). The infection through the respiratory droplet is mostly occurring when an infected person with respiratory symptoms (sneezing, coughing) sings or talks within 1 metre with a non-infected person, thereby transmitting the virus to the non-infected person (Figure 2-2).

Studies have revealed that the virus is also transmitted via aerosols (Tang et al., 2020). Research by van Doremalen et al. (2020) described the SARS-CoV-2 as infectious for hours while in the air. It can be transmitted through aerosol when an infected person exhales the virus droplets, suspended in the air, and generate an aerosol. A noninfected person can inhale the aerosol and get infected, provided the inhaled quantity of aerosol is enough to get the person infected. Johansson et al. (2021) also explained that asymptomatic people are responsible for about 50% of transmission, based on their analysis on the amount of infected people by asymptomatic individuals. Kronbichler et al. (2020) further substantiated that infected people who do not show symptoms are a significant contributor to the spread of the virus in a population, but there is no substantial information about the asymptomatic people with the SARS-CoV-2 (Gao et al., 2020). Furthermore, SARS-CoV-2 can be transmitted from mother to child through contact,

but there is no firm evidence of transmission through breastfeeding of children (Wang et al., 2020). Although the SARS-CoV-2 is evident to have multiple transmission means of contracting the virus, it is not yet known, all the transmission pathways of the SARS-CoV-2 to humans.

Symptomatic and asymptomatic cases of the SARS-CoV-2 are infectious and spread at different levels (Sayampanathan et al., 2021). SARS-CoV-2 appears to primarily be spread through contacts with those who show symptoms and those who do not. Most infected people are symptomatic, while about 40% of infected people do not show symptoms (Yang et al., 2020). The sudden onset of symptoms of infected people with SARS-CoV-2 includes cough, fever, fatigue, headache and loss of smell, as indicated in Figure 2 (Chan et al., 2020; Lai et al., 2020; WHO, 2020e). Some infected people also experience some gastrointestinal symptoms such as vomiting, diarrhea and loss of appetite (Song et al., 2020; Villapol, 2020). Lai et al. (2020) explained further that about half of infected people do not show these symptoms, which might result in patients being diagnosed with another disease. However, diarrhea is associated with a significant percentage of SARS-CoV-2 infections (D'Amico et al., 2020; Wei et al., 2020). In the long term, some cases can become severe, with some infected people exhibiting respiratory diseases such as acute respiratory distress syndrome, an infection that prevents the proper flow of oxygen in the lungs and the entire body (Li et. al., 2020; Zochios et. al., 2020).

Most infected people show mild symptoms, but the virus has a severe consequence on the elderly and people with pre-existing chronic diseases and other medical conditions, having about 17% mortality (Figliozzi et al., 2020; Zheng et al., 2020). The elderly between the ages of 40 and 60 are the most susceptible to the virus with infections in males a bit higher than females, and children infections are uncommon (Lai et al., 2020). Children's infection rates are generally low, but they are not immune to the virus and show mild symptoms (Cao et al., 2020). Studies have shown transmission occurs due to physical contact with symptomatic patients within the domestic environment (Jeong et al., 2020), at work, recreational centres, religious gatherings, public places (Hamner et al., 2020; James et al., 2020; Jang et al., 2020; Park et al., 2020). Also, the proportion of asymptomatic people is expected to vary according to age, based on underlying conditions of older people, with children and young adults being the majority of the asymptomatic patients (Davies et al., 2020). Most studies reported a high rate of asymptomatic patients, while a follow-up showed a significant number of those patients eventually showed symptoms (Byambasuren et al., 2020; Sakurai et al., 2020), with significant percentage of asymptomatic patients never exhibited symptoms of the virus (Wang et al., 2020). Furthermore, several studies described that infections and transmission take place before the carrier shows signs of the virus (Arons et al., 2020; Jang et al., 2020; Tong et al., 2020; Yu et al., 2020). The complexity of the transmission and the continuous spread of the virus explains the difficulty in curtailing the virus, which brings about several local and international measures to curb the spread; thereby reducing the infection and mortality rate.
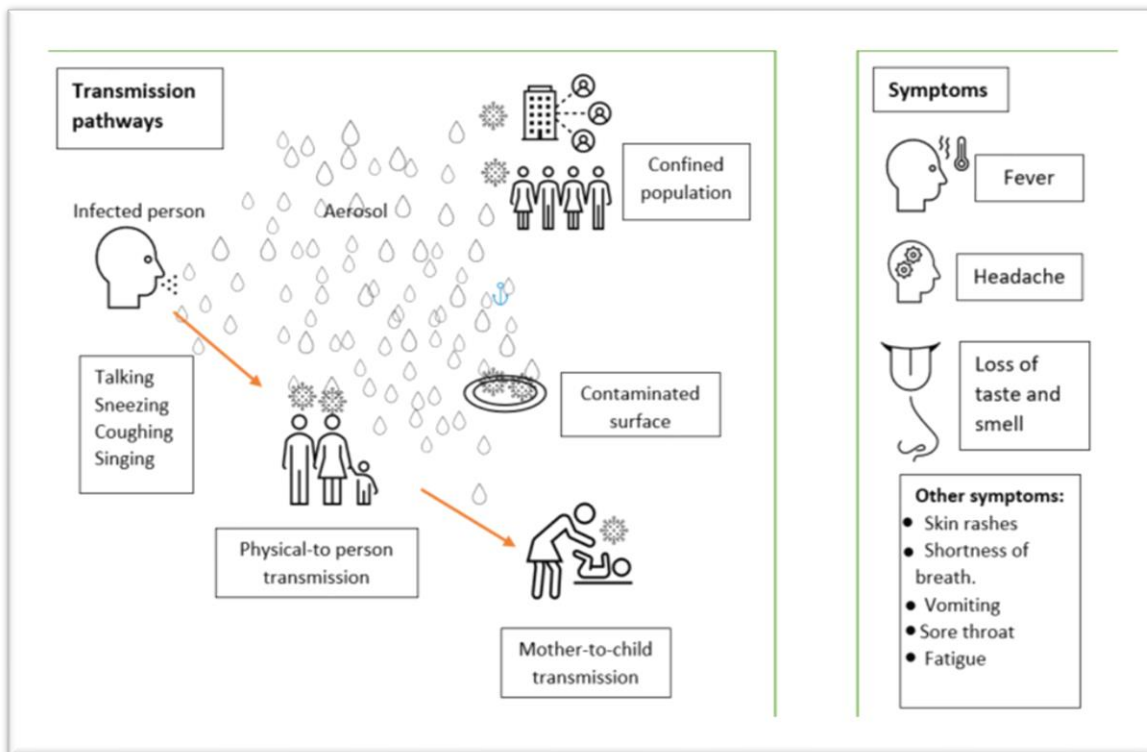
Figure 2-2: SARS-CoV-2 transmission pathways and symptoms adapted by Jeong et al., (2020); Karia et al., (2020); WHO, (2020d)

2.2.1. Factors that accelerate exposure to the SARS-CoV-2

The transmission of the SARS-CoV-2 is possible through multiple means, and several factors contribute to the survival and, eventually, the transmission of the disease (Figure 2-2). These factors can be categorised into human behavioural and environmental factors: social interactions, population distribution, migration, contact with animals, and agricultural development (Barratt et al., 2019). These factors are responsible for the contraction of the SARS-CoV-2, and explains how the virus enters the human body, popularly through contact with the virus droplets, aerosol in the human environment. The WHO explains that it is unknown if SARS-CoV-2 will persist on surfaces, but the virus can survive on surfaces for hours (Parry, 2004). The human behavioural factor, by extension, population distribution, social interaction between people, is significant in contributing to the transmission of the virus through surfaces. The physical interaction of people within a confined space without following guidelines such as physical distancing, use of face mask and washing of hands mostly lead to the presence of the SAR-CoV-2 on surfaces. Also, a lack of proper ventilation with the confines of space contributes to the survival of the exhaled SARS-CoV-2 droplet in the air. This prompt the responsibility of the people to ensure proper hygiene and follow all health guidelines in all areas such as homes, hospitals, and public places to curb the transmission of the SARS-CoV-2. The need to pay attention to all contributory factors to the exposure of the virus would provide a better understanding of measures to control the spread of the virus.

The relationship between environmental factors such as temperature, relative humidity and the prevalence of the SARS-CoV-2 is one of the most important concerns due to the high transmission rate of COVID-19 in the world (Abbasi et al., 2020; Prata et al., 2020). A study by Mecenas et al. (2020) reviewed literatures on the seasonality of the SARS-CoV-2 to describe the relationship between the increase in temperature and relative humidity, and the prevalence of the virus. The study identified that most literature reviewed described that both high and low temperature leads to low infections of the SARS-CoV-2, but the factors are insufficient to determine the variability in the virus prevalence. Also, a study by

Kassem (2020) described the relationship between the number of positive COVID-19 cases and temperature in 43 countries. The association was observed over four time periods from January, February, or March 2020, when the first case was recorded in the countries, till April, May, or July 2020. The study reported an inverse relationship between temperature and the prevalence of the virus, but the significance of the association varies by geography. Temperature is not the most significant factor contributing to the transmission rate of the virus (Notari, 2021). Changes in seasons or weather, such as an increase in relative humidity and temperature, are not enough major factors responsible for a decline in the infection rate of the SARS-CoV-2, but with an improvement and adherence to the public health interventions (Poirier et al., 2020). Generally, the WHO (2020e) reported that low infections of coronavirus could be associated with sunlight, low or high pH and heat. However, research by Chin et al. (2020) described that the SARS-CoV-2 is stable at a pH between 3-10, at 20°c (room temperature), but the virus is susceptible to disinfection of surfaces where they are present.

2.3.   Wastewater Based Epidemiology

Wastewater-based epidemiology is an innovative approach based on the chemical analysis of specific human metabolic excretion products (biomarkers) in wastewater (Lai et al., 2018; Rousis et al., 2017; van Nuijs et al., 2011b), with the potential to act as a complementary approach for current infectious disease surveillance systems and an early warning system for disease outbreaks (Sims & Kasprzyk-Hordern, 2020). These biomarkers could be harmful substances such as human excreta (faeces or urine), food substances that can be traced to a population within the confines of a geographical defined wastewater catchment area (Sims & Kasprzyk-Hordern, 2020). There is an assumption that identifying and quantifying biomarkers in community wastewater reflects the population health status in real time, as they contain rich chemical and biological information (Mao et al., 2020). Wastewater analysis is equivalent to obtaining and analysing a community-based urine and faecal sample. Monitoring temporal changes in virus concentration in combination with monitoring metabolites and biomarkers for population adjustments allows early detection of outbreaks. Additionally, a well organised spatial sampling would enable the discovery of outbreak-prone regions (Xagoraraki & O'Brien, 2020), essentially providing population-scale information on viral infections within catchment boundaries (Choi et al., 2018).

The WBE process encompasses the stages of WBE applicable to all related WBE-based research, including epidemiological public health research (Figure 2-3). The wastewater from a geographically defined population flows into the wastewater treatment plants. The WWTPs serve one or more communities in which the population within the communities contributes to the wastewater in the WWTPs. The wastewater samples are collected from the untreated WWTPs, which in most cases involves a daily collection of samples at different time intervals, and a single daily aggregate of the sample is obtained (Markosian & Mirzoyan, 2019; Sims & Kasprzyk-Hordern, 2020). However, in the Netherlands, wastewater samples are collected twice weekly from over 300 WWTPs, and a weekly aggregate is obtained (RIVM, 2020a). The aggregation of the samples is essential based on the variation of the wastewater flowing into the WWTP. The wastewater samples collected are processed and undergo an analysis involving extraction and concentration of samples for biomarkers. The identification of biomarkers depends on the purpose of the WBE, such as identification of illicit drugs, pharmaceutical products, or viral disease RNA in the wastewater samples. An important consideration in WBE is the flow rate of the wastewater into the WWTP because, in most cases, there is variation of the wastewater flows, mostly caused by seasons (Rashid & Liu, 2020). For example, there are expectations of large influent flows of wastewater during wet seasons due to the effect of rainfall, which would influence the concentration of the samples used in the identification of biomarkers. The

concentration of the biomarker in the wastewater samples and the volume of the wastewater accounts for the load of the biomarkers identified (Zuccato et al., 2008). This step is important in WBE for normalisation of the viral load flowing into the WWTP to correct for the contribution of human waste into the WWTP. Hence, the concentration of biomarker in the wastewater samples requires population normalisation to estimate the population contributing to the biomarker. Population and flow rates normalisation allow comparing biomarkers across different spatial scales and socioeconomic statuses (Bodík et al., 2016; Pereira et al., 2015). Therefore, WBE detects biomarker concentrations in untreated wastewater, uses the inhabitants in the catchment areas and the wastewater flow rates to obtain per capita mass load estimates of the biomarkers to offer community health information (Boogaerts et al., 2021; Lai et al., 2013; van Wel et al., 2016).
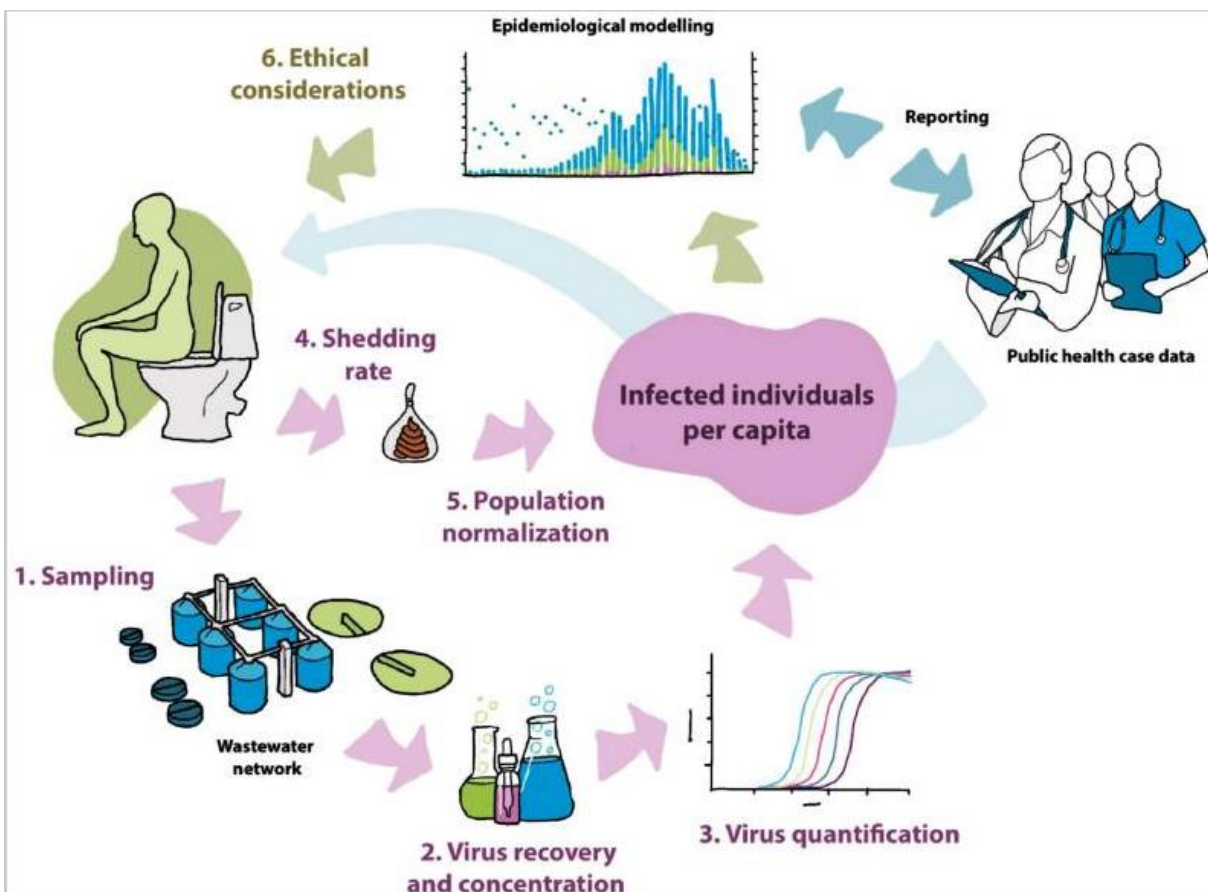
Figure 2-3: An illustration of wastewater-based epidemiology approach for community COVID-19 surveillance as depicted in Polo et al. (2020a)

2.3.1. Wastewater Based Epidemiology and other related studies

WBE is an emerging field that has had tremendous success since Daughton first proposed the idea in 2001, hypothesizing that drug traces in wastewater may be connected back to its usage within a population (Daughton, 2001). Earlier research on WBE were focused on its application to consumption of illicit drugs such as cocaine, methamphetamines, cocaine and heroin (Boleda et al., 2007; Castiglioni et al., 2006; Kasprzyk-Hordern et al., 2008), in which pioneering research by Zuccato et al., (2005) successfully used WBE to investigate cocaine consumption and its quantification in wastewater, when he successfully extracted and measured cocaine in surface water and wastewater in Italy, and assessed the consumption of

cocaine use in the community. Since then, studies worldwide have adopted and established the monitoring initiative of the WBE research in Europe (Thomas et al., 2012), the USA (Halden et al., 2019) and Australia (Lai et al., 2016; O'Brien et al., 2019; Tscharke et al., 2016). With this method, untreated wastewater is considered to store human excretion, which includes illicit drugs metabolites. The concentration of the metabolites at the WWTP could be equivalent to the amount of the metabolites excreted by the catchment population if the excretion remains stable in wastewater. Traditional surveys for estimating illicit drug use consumption can be inaccurate and prone to response bias. WBE has proven to be successful due to the direct quantification of drug residues (Irvine et al., 2011), for comparing population-scale consumption of illicit drugs in different countries (Thomas et al., 2012). Therefore, an important consideration in wastewater-based epidemiology is the estimation of the contributing population to the sampled wastewater (van Nuijs et al., 2011). Choi et al. (2019) illustrated how chemicals from illicit drug consumption in wastewater reflect the respective populations' social, demographic, and economic properties. This highlighted the potential importance of wastewater in understanding sociodemographic determinants of population health. From a temporal perspective, the WBE approach can provide crucial and timely information on short (Karolak et al., 2010) and long-term trends (van Nuijs et al., 2011) in illicit drug use.

WBE has also included a wide range of other self-consciously consumed substances, ranging from those linked to lifestyle choices like alcohol consumption (Reid et al., 2011; Rodríguez-Álvarez et al., 2014; Zheng et al., 2020), psychoactive substances (Kinyua et al., 2015; Reid et al., 2014), nicotine consumption (Senta et al., 2015), caffeine consumption (Driver et al., 2020). A study by Boogaerts et al. (2016) assessed the feasibility of WBE to monitor the spatial and temporal trends in alcohol consumption among various Belgian inhabitants. It highlighted the importance of WBE as a complementary indicator on spatiotemporal trends in alcohol consumption on different scales (i.e., local, national, and international scales). WBE was employed by Driver et al. (2020) to assess alcohol, nicotine and caffeine consumption of a university campus in the U.S.A, to complement the self-reported data available. In this study, alcohol consumption estimation from WBE coincides with the self-reported data, which is expensive to conduct over a long period of time. Other studies also illustrated the association of alcohol dependence (Arria et al., 2011), pharmaceuticals (Baz-Lomba et al., 2016), nicotine (van Wel et al., 2016), caffeine (Senta et al., 2015) and their estimates in wastewater. Hence, the feasibility of WBE in the assessment of these self-assessment consumed substances in a specified population at a low cost for a specified period. Concerning unintentional consumption of chemicals, some studies have extended the WBE approach to assess (Rousis et al., 2017) and measure the effects of exposure to pesticides (Rousis et al., 2016) and heavy metals (Markosian & Mirzoyan, 2019) and their value in providing supplementary information to biomonitoring studies (Rousis et al., 2017).

Waterborne viruses are an important element of biomarkers in wastewater and have been linked to disease outbreaks. An important feature of viruses is that they grow inside the host cells. Therefore, the viral concentration excreted by the human population will correlate with the corresponding concentration in wastewater. Viruses have been identified as one of the most significant pathogens present in wastewater (Toze, 1997). WBE has a long history in public health as a surveillance tool for monitoring the spread of viruses, particularly for human enteric viruses such as hepatitis (Miura et al., 2016b), astroviruses (Prevost et al., 2015), norovirus and rotavirus (Santiso-Bellón et al., 2020). Table 2 summarizes previous studies using WBE to evaluate the population's levels of different substances and viruses. In the underlisted WBE studies on viruses, the viruses have been detected in excreta and wastewater.

Table 2: Summary of WBE studies adapted from Xagoraraki and O'Brien (2020)

Substances/Viruses	Authors
Adenovirus	(Carducci et al., 2008; A. Hata, Kitajima, & Katayama, 2013; Rosa, Pourshaban, Iaconelli, & Muscillo, 2010)
Amphetamine	(Centazzo, Frederick, Jacox, Cheng, & Concheiro-Guisan, 2019; Irvine et al., 2011)
Astrovirus	(Le Cann, Ranarijaona, Monpoeho, Le Guyader, & Ferré, 2004; Le Guyader et al., 2008; Logan, O'Leary, & O'Sullivan, 2007)
Doxepin, Verapamil, Tranquilizers, Opiates	(Hummel, Löffler, Fink, & Ternes, 2006)
Enterovirus and norovirus	(A. Hata et al., 2013; Hewitt, Leonard, Greening, & Lewis, 2011; Katayama et al., 2008; Le Guyader et al., 2008)
Hepatitis A virus	(Morace et al., 2002; Prado et al., 2011)
Hepatitis E virus	(Masclaux, Hotz, Friedli, Savova-Bianchi, & Oppliger, 2013; Orrù et al., 2004)
Influenza	(Heijnen & Medema, 2011; To et al., 2010; Vester et al., 2010)
Ketamine and methamphetamine	(Gao et al., 2020)
Norovirus	(Hewitt, Greening, Leonard, & Lewis, 2013; Hewitt et al., 2011; Katayama et al., 2008)
Opoid	(Endo et al., 2020; Gushgari, Venkatesan, Chen, Steele, & Halden, 2019)
Polyomavirus	(Bofill-Mas, Pina, & Girones, 2000; Kitajima, Iker, Pepper, & Gerba, 2014; Siebrasse et al., 2012)
Rotavirus	(Kitajima et al., 2014; Prado et al., 2011; Sedmak, Bina, MacDonald, & Couillard, 2005)
Salivirus	(L. Li et al., 2009; Nielsen, Gyhrs, Nielsen, Pedersen, & Böttiger, 2013)
Sapovirus	(A. Hata et al., 2013; Kitajima et al., 2014)

2.3.2. Wastewater Based Epidemiology in the surveillance of the SARS-CoV-2

Since one of the first reports of SARS-CoV-2 in human waste (Holshue et al., 2020), globally, a growing number of studies have tested for the presence of SARS-CoV-2 RNA in treated untreated and/or urban wastewater and wastewater treatment plants (WWTPs) to monitor the spread of the virus (Zahedi et al., 2021), further supporting the possibility of the WBE approach. The WHO stated that research on WBE should be viewed as a significant public health goal in order to advance information about COVID-19 (Polo et al., 2020; WHO, 2020d), but an important consideration in research on WBE is the comparison of the virus concentration in wastewater with the other virus surveillance indicators, thus providing an unbiased response to key aspects on the prevalence of the SARS-CoV-2 infections within a community to assist public health professionals in making informed decisions (Bivins et al., 2020). The application of WBE as an epidemiological technique for SARS-CoV-2 prevalence in a community is feasible regardless of whether the virons are infective in wastewater (Wurtzer et al., 2020). The wastewater sampling procedure is as shown in figure 2-3, describes the wastewater sample collection where a single sample collected gives the infection of the SARS-CoV-2 within the population. Thus, the sampling procedure as a strategy for WBE is useful as a potential prediction tool for the prevalence of the SARS-CoV-2 virus and, consequently, tackling the COVID-19 pandemic. The wastewater concentration data are combined with other epidemiological data for precise modelling that allows the prediction of the SARS-CoV-2 in different communities.

Globally, many countries have reported the presence of SARS-CoV-2 RNA in wastewater, providing additional information on viral circulation along the lockdown assessments and pandemic phases (Aguiar-Oliveira et al., 2020). At different stages of the pandemic, WBE is being implemented as studies have shown that the SARS-CoV-2 RNA is often present in patient stool samples and wastewater in Italy (La Rosa et al., 2020; Rimoldi et al., 2020), The Netherlands (Lodder and de Roda Husman, 2020; Medema et al., 2020), India (Kumar et al., 2020), Spain (Randazzo et al., 2020), Japan (Haramoto et al., 2020) and the USA (Sherchan et al., 2020; Wu et al., 2020). These studies have established their research finding of the presence of the SARS-CoV-2 RNA in faeces with some reports on the relationship between the SARS-CoV-2 wastewater RNA concentration and the positive tested cases; nonetheless, the different countries are thereby incorporating WBE into their national and regional SARS-CoV-2 surveillance programmes to detect the virus outbreak and provide early warning of the virus spread (WHO, 2020d). The presence of the SARS-CoV-2 RNA in wastewater reveals the potential of wastewater-based epidemiology (WBE) in the surveillance of the SARS-CoV-2 with the establishment of its relationship with clinical testing indicators (Nemudryi et al., 2020).

In the Netherlands, Medema et al. (2020) suggested that WBE can act as a sensitive and pre-emptive system in understanding the circulation of the virus in the population and subsequently support social distancing policies. The study findings detected virus circulation in sewage six days before the first case was reported, and as the cases increased, there was a significant correlated increase in virus particles in the wastewater. Similarly, using samples from untreated WWTP in Montana, USA, Nemudryi et al. (2020), over a 74 day time period, showed SARS-CoV-2 RNA levels in wastewater followed symptom onset but precede clinical PCR test results. In the study by Wu et al. (2020b), unlike in Ahmed et al. (2020b), there was a significantly higher concentration in virus particles than expected compared to the clinically confirmed cases in Massachusetts. The research was based on significant uncertainty such as viral load in the stool of positive patients and therefore was subject to additional experiments across different spatial units to calibrate the numbers. Nonetheless, this study can inform quarantine efforts and social distancing decisions. Saguti et al. (2021) suggested collecting and analysing the SARS-CoV-2 wastewater RNA concentration across multiple regions to enable effective contact tracing and specific restriction measures. Therefore, in this study, the SARS-CoV-2 spatial and temporal spread across multiple regions is explored in comparison with other epidemiological indicators to describe the potential of the WBE approach to monitoring the virus. Table 3 highlights the summary of recent research on WBE that indicates the feasibility of monitoring the SARS-CoV-2 and those that have explored the relationship between WBE and a few epidemiological indicators.

Table 3: Summary of recent studies on WBE that indicate the feasibility of monitoring the SARS-CoV-2. Adapted from (Zhu et al., 2021)

Authors	Country/region	Sampling Period	Findings
Agrawal et al. (2021)	Frankfurt, Germany	June to September 2020	The SARS-CoV-2 RNA was detected in wastewater. Identification of resurgence of the virus in the communities through the SARS-CoV-2 wastewater RNA and the increase in the virus RNA in wastewater precedes the averaged positive cases.
Hata et al. (2021)	Ishikawa, Japan	March 2020 to April 2020	When the SARS-CoV-2 prevalence was less than one positive case per 100,000 inhabitants, the wastewater samples tested positive for the virus.
Kaplan et al. (2020)	Hamden, East	February to April	The research detected SARS-CoV-2 in the

	Haven, New Haven, Woodbridge in Connecticut, U.S.A.	2020	municipal wastewater and reported that the SARS-CoV-2 RNA in wastewater at the municipalities precedes the hospital admissions by an average of 3 to 5 days. The research also suggested that over 80% of the infection risk population were removed due to stay-at-home restrictions.
La Rosa et al. (2020)	Milan, Italy	February 2020 to April 2020	Wastewater samples tested positive for the SARS-CoV-2 but have a negative correlation with the infections. The number of infections was low, but the samples were positive.
Nemudryi et al. (2020)	Bozeman, Montana, USA	March 2020 to June 2020	The onset of the symptoms of SARS-CoV-2 precedes the virus RNA concentration in wastewater by 5-8 days. While the wastewater viral RNA precedes the positive cases conducted in the laboratory test by 2-4 days.
Prado et al. (2021)	Niterio, Rio de Janeiro Brazil	April to August 2020	The detection of the SARS-CoV-2 RNA has higher detection rates and is effective in the surveillance of the virus — identification of the strain of the virus circulating the whole in wastewater.
Peccia et al. (2020)	New Haven, Connecticut, USA	March 2020 to June 2020	The SARS-CoV-2 RNA levels in wastewater precede the positive cases for COVID-19 by about two days and reported hospital admissions by about four days.
Saguti et al. (2021)	Gothenburg, Sweden	February to July 2020	The SARS-CoV-2 wastewater RNA should be monitored as it proves to be early surveillance of the virus outbreak. It precedes the number of hospital admissions by 19-21 days.
Weidhaas et al. (2021)	Utah, U.S.A.	April and May 2020	The detection of the SARS-CoV-2 RNA in about 60% of the wastewater samples. The increase in the virus concentration in wastewater positively correlate with the outbreak of the COVID-19 in the communities sampled. Still, the decline in the wastewater RNA lags the decline in the positive cases.
Wurtz et al. (2021)	Marseille, France	July to December 2020	The research identified the presence of the SARS-CoV-2 RNA in wastewater. It explained a significant correlation between the number of positive cases and the virus RNA concentration in wastewater.

2.4. Application of spatial and statistical methods from epidemiological studies

Different studies have employed spatial and statistical techniques in the understanding of the spatiotemporal analysis of COVID-19 and the influence of environmental variables on the dynamics of the SARS-CoV-2. Several methods are applied in studies relevant to COVID-19 with diverse application to different research themes such as spatial and temporal pattern analysis of COVID-19, environmental

variable influences, population health surveillance, and web-based spatial and statistical analysis. Since this study focuses on employing methods for the spatial and temporal analysis, Table 4 shows different studies that employed geospatial and statistical methods for the spatiotemporal analysis of COVID-19.

Table 4: List of methods of analysis used in different COVID-19 related studies and other epidemiological studies.

Study	Methods used	Data used	Analysis
Ahmadi et al. (2020)	Pearson correlation and Sobol'-Jansen methods	COVID-19 Infected cases, population density, solar radiation, humidity, wind speed, and intra provincial movement	The COVID-19 infection rate is directly correlated with the population density, solar radiation, and intra-provincial movement.
Andrade et al. (2020) Hohl et al. (2020)	Space-time scan statistics	COVID-19 Confirmed cases	Detection of active and emerging Spatiotemporal clusters of the SARS-CoV-2. Detection of county-level daily clusters of COVID-19.
Baker et al. (2020)	Global Model development	COVID-19 confirmed cases, humidity, and temperature	The research conducted a geospatial analysis to visualize the spatial pattern of the COVID-19 confirmed cases at different instances. The authors explained that the temperature during summer might not affect the increase in infections of the virus.
Bavia et al. (2020)	Pearson correlation, Cross-correlation function	Dengue cases, demographic data (income, temperature, precipitation	The research analyzed the spatial and temporal distribution of positive dengue cases. It describes the temporal relationship between precipitation, temperature, and dengue cases. It also illustrates the spatial relationship between the positive dengue cases and income.
Gross et al. (2020)	Space-time statistics	COVID-19 Confirmed cases	The spatial and temporal propagation of the SARS-CoV-2 infections in different cities indicated a constant infection rate in the early stages of the virus and an exponential decrease in infection at a later period because of the mitigation measures.
Gupta et al. (2020)	Geospatial analysis using ArcGIS	COVID-19 cases, temperature, and humidity	The study describes the effects of weather parameters on the prevalence of the SARS-CoV-2 using GIS to describe the spatial spread of the virus at different time steps.
Huang et al. (2020)	Logistic Regression model and Susceptible exposed infectious removed (SEIR) model	COVID-19 reproductive number and cumulative confirmed cases	Spatial and temporal analysis of COVID-19 features which discovered the large gathering from Hubei province into Wenzhou caused the outbreak of the virus.
Xiong et al.	Pearson correlation	COVID-19 Confirmed	Significance of influencing factors and

(2020)	and geospatial statistics	cases	spatial autocorrelation of the factors contributing to COVID-19 spread
Zhang et al. (2020)	Moran's I and Local indicators of spatial association (LISA)	COVID-19 and SARS confirmed cases	Comparison of COVID-19 and SARS incidence with COVID-19 with higher incidence. Identification of clusters of COVID-19 and the significance of clusters.

2.4.1. Geospatial analysis of spatiotemporal patterns of disease transmission

The COVID-19 epidemiological indicators all have spatial dimensions and temporal dynamics, which facilitates mapping the spatio-temporal patterns of the virus prevalence across multiple spatial scales (Franch-Pardo et al., 2020). To map the spatio-temporal patterns, understanding COVID-19 space-time dynamics is vital to define the extent of the variation of the virus, its impact, and mitigation measures (Coura-Vital et al., 2021; Masrur et al., 2020). Andrade et al. (2020) and Hohl et al. (2020) employed the space-time cluster techniques to explore the spatial and temporal distribution of COVID-19 cases across the municipality and county level in Brazil and the U.S.A, respectively. Using ArcGIS, they modelled the increase in COVID-19 cases to determine spatial clusters and hotspots of the SARS-CoV-2 virus. Desjardins et al. (2020) and Kulldorff et al. (1998) explained that the space-time Poisson scan technique is a suitable spatiotemporal statistical technique for primarily identifying the significant clusters of epidemiological variables such as disease incidence and deaths. This research aims to identify spatial and temporal patterns of SARS-CoV-2 wastewater data and the epidemiological at different time series across different geographical scales. The visualisation of spatial patterns through different indicators requires real-time mapping hosted on a geovisualisation platforms (Boulos & Geraghty, 2020) or time-series mapping at different geographical scales (Patel et al., 2007). The concept of geovisualization involves the creation of an online platform such as dashboards and spatial applications suitable for describing the relationship between variables of different in real-time (Jing et al., 2019). The time-series mapping is applicable to this research to generate the spatial patterns of the SARS-CoV-2 incidence across different spatial scales.

Zhang and Schwartz (2020) employed a GIS-based spatial analysis using ArcGIS to visualise COVID-19 cases and deaths at different times and describe the patterns across the different geographical units in the U.S.A. The results show that areas close to metropolitan cities have high numbers of SARS-CoV-2 infections based on visual interpretation and comparison of the spatial patterns. Some small geographical areas record similarly high incidences as in big cities. The same study used a regression model to investigate the risk factors to infection and mortality risk factors with inputs such as the COVID-19 positive cases, deaths, and socioeconomic factors such as population density and population distribution across age groups. Mapping the SAR-CoV-2 incidence across a fine spatial scale like the county level indicates a detailed spatial pattern. Zuo et al. (2020) employed GIS-based spatial analysis for time-series mapping of tuberculosis incidence between 2004 to 2017, based on the spatial autocorrelation results across different geographical units in China. The study identifies the areas with a high incidence of tuberculosis and the differences across the different geographical units of analysis. Yue et al. (2019) also explored the seasonal patterns of dengue fever cases using ArcGIS spatial analysis extension, generating time series maps that indicate the yearly spatial occurrence and spatial diffusion of the dengue fever across different provinces and regions in China. They concluded that the visualisation of the dengue fever's epidemiological dynamics is helpful to the health authorities to make informed decisions on plans and measures to control the fever outbreak.

2.4.2.   The statistical relationship between epidemiological indicators

The exploration of statistical relationships between different indicators such as educational, socioeconomic and epidemiological indicators explain the statistical significance and provides an intuitive understanding of the underlying associations between these indicators (Gunawardane et. al., 2009). The statistical relationship between indicators of different temporal trends can be established using various statistical tools such as regression model, correlation analysis, and causality model (Bhaskaran et. al., 2013). Similarly, a correlation analysis of indicators in epidemiological studies provides an understanding of the association of the variables with more insight on the trends obtained from the descriptive analysis. Correlation is used to explain the relationship between two quantitative variables, with the correlation coefficient is used to measure the degree of association (Taylor, 1990). The assumption is for the relationship between the variables to be linear, which means as one variable increases or decreases, the other variable also increases or decreases. In this thesis, the correlation between the trends in the SARS-CoV-2 wastewater concentration and the trend in the individual epidemiological indicators is explored with the assumption that these variables have a linear relationship.

The regression model is another technique used to obtain the relationship between the dynamic of a variable with another. Regression analysis is a prediction technique that explains if one variable's variation leads to variation in another variable through the association between dependent and independent variables (Crawford, 2006). A study by Wang et al. (2021), employed multiple linear regression and Spearman correlation analysis to investigate the relationship between the positively confirmed cases of COVID-19 and the influencing factors of the virus. They used the Spearman correlation technique to describe the association between the influencing factors such as temperature, precipitation, carbon monoxide and particulate matter and the COVID-19 confirmed cases, and multiple linear logistic regression to investigate the lag effect of the most significant COVID-19 influencing factor. A study by Gupta et al. (2020) employed linear regression analysis to determine the relationship between the increased rate of spread of COVID-19 and weather variables in the USA. The study explains a positive correlation between the rate of increase in the COVID-19 cases and temperature, but a negative correlation between the number of cases and humidity. They established that no conclusion should be drawn from the result due to a low correlation value and suggested other methods such as the Pearson correlation analysis. Hence, regression analysis can describe the relationship between indicators and develop a prediction model for the variables. But correlation analysis has been proposed in different studies as a suitable technique that offers a unitless measure of association to describe the statistical significance of indicators in epidemiological studies.

Cebrecos et al. (2018) established the statistical relationship between cardiovascular disease incidence and socioeconomic deprivation across three geographic scales. They employed the Pearson correlation analysis to describe the relationship between socioeconomic deprivation and cardiovascular diseases across the three distinct administrative units in Spain. Their result records positive correlation and statistical significance across the geographical units. They also explained that there is a correlation between the variables and administrative units. Likewise, the statistical association result validates the geographical stability analysis between socioeconomic deprivation and cardiovascular disease. They highlighted that using statistical methods and the Pearson correlation technique effectively explained the association between the variables and thus a suitable technique across different scales. Therefore, this demonstrates that the bivariate Pearson correlation analysis can be suitably employed to describe the statistical association between SARS-CoV-2 wastewater RNA concentration trends and the epidemiological indicator across different spatial units.

The spatial analysis of the statistical relationship between the indicators provides more insight into the correlation variation across space based on the spatial unit of analysis (Patel et al., 2007). Since the statistical analysis provides a result of the significance between the trends and the indicators, then a spatial analysis would help visualise the spatial pattern of the correlation analysis. Briz-Redón and Serrano-Aroca (2020) and Wang et al. (2021) used ArcGIS to generate series of maps to visualise the spatial autocorrelation characteristics of the COVID-19 positive confirmed cases, which shows the areas of high and low significance. Therefore, ArcGIS will be used to produce maps showing correlation patterns between variables across different geographical scales.

2.4.3.   Temporal relationship between epidemiological indicators

Several analyses have been used to depict the temporal relationship between epidemiological indicators. For example, Mirri et al. (2021) studies on the possible role of particulate matter (PM) distribution on the spread of SARS-CoV-2 used the Granger causality method to analyse the PM concentration time series the COVID-19 confirmed cases. The result indicates a correlation between the indicators with a time lag period of 14 days. The method was validated using a machine learning algorithm, and the authors suggested that other methods can be employed to corroborate the association between the time series of the indicators. Also, Benvenuto et al. (2020) used the Auto-Regressive Integrated Moving Average (ARIMA) model to predict COVID-19 incidence and prevalence trends. They suggested that the model requires more data with a high temporal resolution for a detailed prediction result because the modes of the data influence the prediction. Although ARIMA models describe the underlying trends and the variation of frequency in a time series, they are not suitable for time series with points that had been increasing reverses and decreases (Schaffer et. al., 2021). Wang et al. (2020) also predict the epidemiological trends of SARS-CoV-2 in China by analyzing the time series of the positive cases and mortality using the prediction models such as ARIMA model and α-Sutte indicator. They compared the prediction indices of the two methods and concluded that the ARIMA model has a higher prediction error compared to α-Sutte indicator and suggested other regression techniques can be employed.

Bhaskaran et al. (2013) used the Poisson regression technique to explore the relationship between time series of exposure such as weather indicators, air pollution or pollen and health outcomes such as incidence, mortality, hospital admission. The results describe that the change in the health outcome is influenced by a change in the exposure and suggested another prediction method that would describe the relationship between two time series of individual health outcome variable and exposure can be employed. Bavia et al. (2020) used the cross-correlation function (CCF) to describe the temporal relationship between time series of dengue incidence and weather effects such as precipitation and temperature in Southern Brazil. The result explains the time lag between dengue incidence and precipitation and, subsequently, dengue incidence and temperature. Also, a study by Huang et al. (2011) used CCF to illustrate the relationship between the time series of malaria incidence and some meteorological indicators such as rainfall, relative humidity and temperature. Furthermore, the authors explain that the CCF technique is suitable to identify short time lag between two time series. Therefore, in this study, CCF analysis will be used to describe the relationship between two time series of SARS-C wastewater RNA concentration and selected epidemiological indicators.

3. METHODOLOGY

The purpose of this chapter is to describe the general overview of the methodological workflow, research design, selection of the epidemiological indicators, spatial units of analysis, the selected study area, methods, and tools employed in this thesis. The research focuses on a comparative analysis of the SARS-CoV-2 concentration in wastewater and its relationship with other epidemiological indicators.

3.1. Overview of the research

The general overview of the methodological workflow and approach is as shown in *figure 3-1* below. The research methodological approach has different phases, and the methods utilized for this study are in four different stages. The research commenced by reviewing literature on the background of SARS-CoV-2 as causative of COVID-19, the state of the art of the virus globally and in the Netherlands and the formulation of the introduction to the research. Also, the research methods employed in this research involve three interconnected approaches to uncover and explain the relationship between the SAR-CoV-2 wastewater RNA and other epidemiological indicators related to COVID-19.

The first approach involved the acquisition, preparation, and organisation of the epidemiological indicators for spatial and temporal analysis. Upon reviewing links of available data sources of epidemiological indicators, the datasets of the epidemiological indicators were downloaded. The data preparation including database management was based on queries by location/attributes, check for data completeness and exploration of spatial attributes of the data. The attributes of the downloaded dataset were reviewed to select suitable epidemiological indicators. These steps were necessary to bring the data together and suitably organize them for temporal and spatial analysis.

The second approach commenced with the review of literature for a suitable spatial and temporal analysis of disease trends. To analyse the temporal patterns of the SARS-CoV-2 wastewater RNA concentration and other epidemiological indicators in pairs, for example, SARS-CoV-2 wastewater data and reported positive cases, SARS-CoV-2 wastewater data and the number of hospital admissions, the trends in these variables were developed using the R statistical software. Then, the organised datasets of the wastewater RNA concentration and the selected epidemiological indicators were spatially and temporally analysed, and the variation of the SARS-CoV-2 across different spatial units in the Netherlands was described using ArcGIS.

The third approach involved the application of statistical correlation analysis to describe the significance of the relationship, and association between the epidemiological indicators. A spatial analysis using ArcGIS to visualize and interpret the relationship between the correlation results of the epidemiological indicators was carried out. Also, the cross-correlation function was employed to explain the relationship between the temporal trends of the SARS-CoV-2 wastewater RNA concentration and the epidemiological indicators. The technique was used to model the relationship between the time series of the SARS-CoV-2 wastewater RNA concentration and the other epidemiological indicators and to identify the time shift between the variables. The time graphs of the time-step analysis and the spatial analysis of the cross-correlation results describe the shift in time and its statistical significance between SARS-CoV-2 wastewater data and the selected epidemiological indicators at the municipality scale, while the spatial analysis of the time lag results shows the variation of the lags across different municipalities.
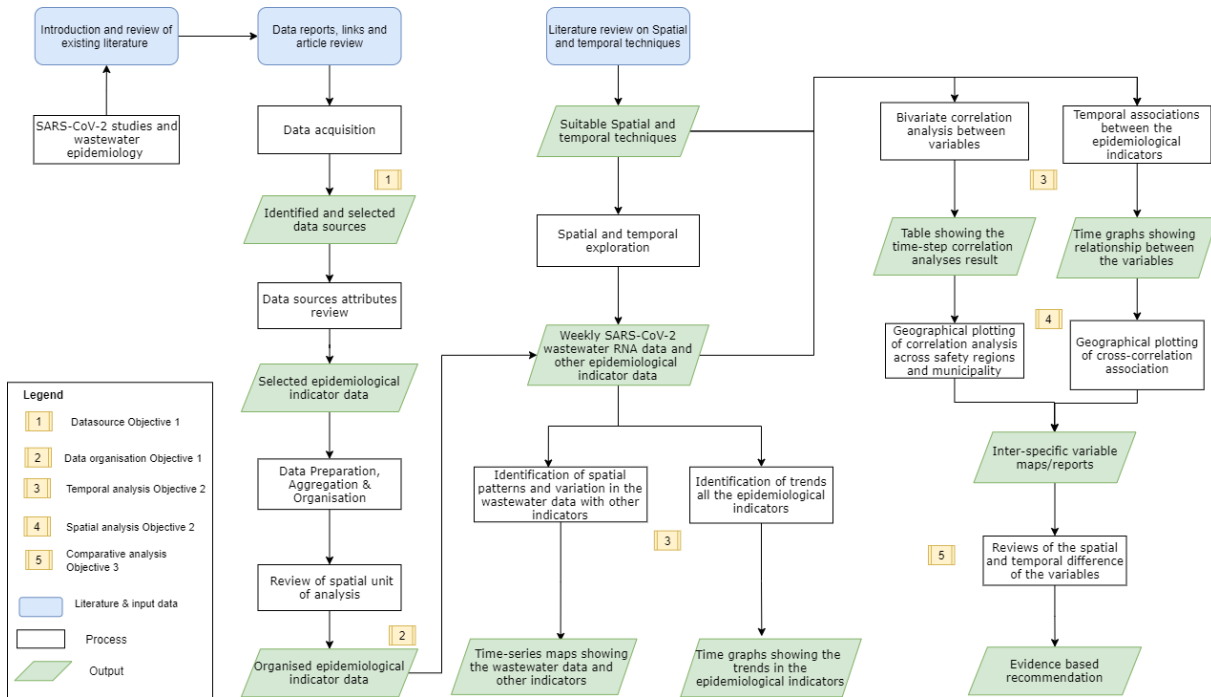
Figure 3-1: Research design and methods workflow

3.2.   Review of the SARS-CoV-2 epidemiological indicators

The epidemiological data used for this research comes from different data sources available through the Dutch Coronavirus Dashboard developed by the Ministry of Health, Welfare and Sport in the Netherlands, RIVM (2020a). The dashboard shows updated information on SARS-CoV-2 in the Netherlands through surveillance of the virus with different epidemiological indicators (Figure 3-2).
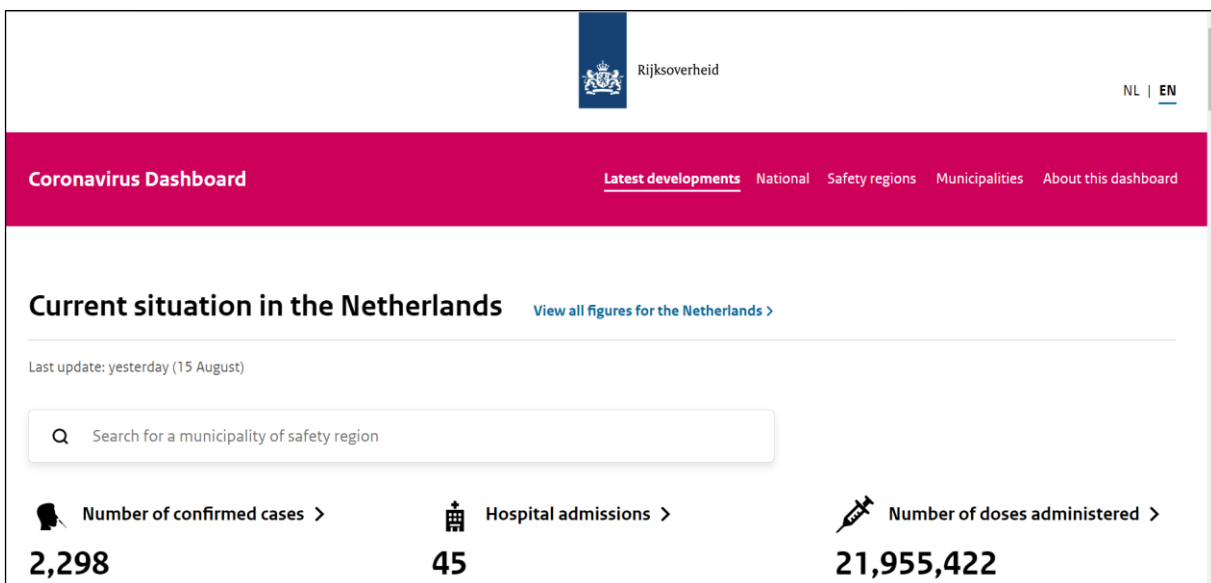
Figure 3-2: Coronavirus dashboard in the Netherlands adapted from RIVM (2020b)

The data used to process the information on the dashboard was made available by the National Institute for Public Health and the Environment, hospitals, and the municipal health services (GGD). Table 5 below provides the summary of the epidemiological indicators contained on the coronavirus dashboard. The highlighted sections in the table are the selected epidemiological indicators and datasets employed for this research.

Table 5: Overview of research data, including the epidemiological indicators on the coronavirus dashboard adapted from RIVM (2020a)

Measures	Indicators	Description	Area of unit	Data availability and sources
Infections	Confirmed cases	The number of positive confirmed cases by municipality and regional level.	Municipality and safety region	Open data available at the municipality and regional level by RIVM
	Infectious people	It gives the number of people that can infect others with SARS-CoV-2. Though, the time period within which one can infect another person differs from one person to another. It uses a range of data sources to determine the number of infected people in the Netherlands.	National	Open data available on a national scale by RIVM
	Number of COVID-19 deaths	It describes the number of people reported death due to the SARS-CoV-2.	Municipality and safety region	Open data available at municipality and region by GGD and RIVM
	Reproduction number	It gives the average number of infected person(s) by at least one person with the SARS-CoV-2.	National	Open data available at national scale reported by RIVM
Hospitals	Number of COVID-19 hospital admissions	It provides daily data on the people infected with the SARS-CoV-2 that are admitted into the regular unit and intensive care units of the hospitals.	Municipality and safety region	Open data available at municipality level collected by the National Intensive Care Evaluation Registry (NICE), Landelijk Coordinatiecentrum Patienten Spreiding (LCPS) and reported by RIVM.
	Number of COVID-19 Intensive Care Units (ICU) admissions	It provides daily data on the people infected with the SARS-CoV-2 admitted into the ICUs of the hospitals. It involves all Dutch patients' ICU admission, including those in German hospitals.	National	Open data available at a national scale reported by RIVM and published by NICE and LCPS.
	Sewage water	The data provides the number of	WWTP level	Open data available

Early indicators	examination	SAS-CoV-2 particles present in different wastewater treatment plants (WWTP).	and safety region	for the different WWTP at safety regional level published by RIVM.
	Symptoms at general practitioners	A weekly data which includes the number of patients with SARS-CoV-2 symptoms complaints to the general practitioner. The data complement the COVID-19 daily reports of cases, hospital admissions and deaths.	National	Open data available at national scale published by Nivel.
Vaccination	Vaccine doses administered	The number of vaccine doses administered to the different vaccine locations in the municipalities. The municipal health services provide the figures.	Not available	Open data not yet available.
Vulnerable groups	COVID-19 cases in nursing homes	It gives the number of reported COVID-19 cases in nursing homes in the Netherlands available at the safety regional level. The data contains the numbers of nursing home residents tested positive and deceased due to the SARS-CoV-2.	Safety region	Open data available at safety regional level reported by RIVM. Reported by the GGD
	COVID-19 cases in disability care	The data provides the number of reported COVID-19 cases in disability care homes. It contains the number of residents of a disability care home tested positive or deceased due to the SARS-CoV-2.	Safety region	Open data available at safety regional level published by RIVM provided by the GGD.
	People over the age of 70 living at home	The data contains the number of people 70 years and above tested positive and deceased to the SARS-CoV-2.	Safety region	Open data available at safety regional level published by RIVM provided by the GGD.
Population	Population per municipality	The data contains the population of the inhabitants in individual municipality in the Netherlands.	Municipality	Open data published by CBS Statistics.
Municipality boundaries	Boundaries of municipalities, provinces, and national boundaries	Shapefiles of the municipalities, provinces, and the national boundary of the Netherlands.	Municipality, Province and National boundary	Open data published by CBS statistics.

3.2.1. Selection of SARS-CoV-2 epidemiological indicators

For this research, SARS-CoV-2 epidemiological indicators datasets were selected based on their temporal resolution, spatial availability, and suitability compared with the SARS-CoV-2 wastewater RNA concentration. The selection of epidemiological indicators to compare with the SARS-CoV-2 wastewater data is an essential step in explaining the SARS-CoV-2 wastewater RNA concentration's potential and describing the trends in the SARS-CoV-2 (Kaplan et al., 2020; Wurtz et al., 2021). Similarly, the

epidemiological indicator(s) that emulate the virus intensity within a population are a precondition for considering the SARS-CoV-2 wastewater RNA concentration as a potential early surveillance tool of COVID-19. The selected epidemiological indicators contain the same temporal resolution and can be aggregated to the same spatial units of analysis. The assessment of the relationship between SARS-CoV-2 RNA in wastewater and the epidemiological indicators requires an agreeable and extended time period to explain the trends in SARS-CoV-2 RNA concentration and the epidemiological indicators (Weidhaas et al., 2021). A study of the SARS-CoV-2 wastewater RNA concentration over a long period offers the observation of the virus signals in a population compared to the positive and active cases (D'Aoust et al., 2021). Agrawal et al. (2021) further state that a long time period of analysis of the SARS-CoV-2 RNA in wastewater provides a broad explanation of the dynamics of infections within a population. Therefore, the epidemiological indicators with the same time period as the SARS-CoV-2 wastewater RNA data was one of the considerations for selecting the epidemiological indicators in this study.

Recent studies that addressed the potential of WBE have explored its relationship with some epidemiological indicators such as the COVID-19 positive cases, hospital admissions and COVID-19 deaths (D'Aoust et al., 2021; Róka et al., 2021; Saguti et al., 2021; Wu et al., 2020). The COVID-19 confirmed cases, number of hospital admissions, deaths caused by COVID-19 are suitable epidemiological indicators in monitoring the dynamics of the SARS-CoV-2 epidemiological scenarios (García-Basteiro et al., 2020; Patel et al., 2020). Varela (2020) further explained that the COVID-19 confirmed cases, number of hospital admissions and deaths, when combined with other useful indicators provide a comprehensive assessment of the trends in the SARS-CoV-2 across different spatial units including national, regional and local units. The combination of these different indicator is essential to understand the problem posed by SARS-CoV-2 across different levels of the health systems (Elliot et al., 2020). Hence, this study employed COVID-19 positive confirmed cases, number of COVID-19 hospital admissions, and number of COVID-19 deaths.

There is a high rate of ICU admissions among patients infected with the SARS-CoV-2, ranging from 3% to 100% of the positive confirmed cases (Arabi et al., 2020; Rodriguez-Morales et al., 2020; Xiaobo et al., 2020). Although there is a variation in COVID-19 infected people hospitalized, about 25% of individuals admitted into hospitals for COVID-19 require ICU admission (Huang et al., 2020b; Xiaobo et al., 2020; Young et al., 2020). Hence, a decline in the ICU admissions of COVID-19 infected patients is equivalent to a decline in hospitalization. Also, the availability of ICU beds is a significant consideration in the variation of COVID-19 mortality rates and complications between different countries, which explains the significance of ICU admissions as a means of surveillance of infection of the virus (Vanhems et al., 2021). Abate et al. (2020) further explained that ICU admissions and ICU mortalities are important indicators to monitor the dynamics of SARS-CoV-2 infections.

The research considers four epidemiological indicators identified on the coronavirus dashboard, which are available in different data sources. These epidemiological indicators include COVID-19 positive confirmed cases, numbers of COVID-19 hospital admissions, COVID-19 ICU admission and COVID-19 deaths. The research explored the trends in these epidemiological indicators and the SARS-CoV-2 RNA wastewater concentration to explain the relationship between these indicators. This was done in order to describe the potential of the WBE approach as a measure of the spatial and temporal variation of the SARS-CoV-2. The selection of reliable the suitable epidemiological indicators for the spatial and temporal analysis of the SARS-CoV-2 requires consistency when exploring the evolution of the disease with these indicators (García-Basteiro et al., 2020). Hence, the exploration of the relationship between these epidemiological indicators and the SARS-CoV-2 RNA wastewater concentration gives a further selection of the epidemiological indicator suited for the correlation and temporal analysis of these epidemiological

indicators. The description of the attributes of the epidemiological indicators downloaded from their different data sources employed in this study is explained below. These epidemiological indicators are available at the different geographic scales as described in Table 5.

3.2.1.1. SARS-CoV-2 wastewater RNA concentration

The RIVM (2020a) has been monitoring the wastewater in the WWTPs in the Netherlands for the SARS-CoV-2 to provide early surveillance of the virus prevalence at different population levels. The monitoring of virus in wastewater by the RIVM has been implemented in collaboration with the Dutch Water Boards. It involves taking a weekly average of wastewater samples from 318 WWTPs across the Netherlands. These wastewater samples are analyzed for the presence of the SARS-CoV-2 RNA in the samples from the different WWTP. The virus particles RNA obtained are then normalized for the flow rate of the wastewater into the treatment plant and the catchments of the populations contributing to the different WWTP. The SARS-CoV-2 wastewater RNA concentration data includes a .csv file with columns such as the date the measurement was taken, the location of the wastewater treatment plant, including the x and y coordinates. It also includes the postal code, the safety region code, safety region name, the amount of RNA per milliliter in the wastewater treatment plant and the RNA flow per 100000 inhabitants.

3.2.1.2. COVID-19 reported positive cases

The evolution of the SARS-CoV-2 has been monitored through testing symptomatic people for the virus RNA and recording the number of infected people (Dong et al., 2020). The number of COVID-19 positive cases is the most important indicator to monitor the SARS-CoV-2 infections appropriately (Sánchez-Romero et al., 2021). In the Netherlands, the number of positive cases confirmed for the SARS-CoV-2 is one of the indicators for monitoring the infections of the virus (RIVM, 2020b). The positive confirmed cases involve daily records of the people testing positive for the virus published by the RIVM, involving positive test reports from doctors and different test laboratories in the country. The positive test report from the laboratories would be accepted only if the tests meet the RIVM standards. The positive confirmed cases data downloaded is a .csv file containing the total daily reported cases, including the date of publication, municipality name and code, province, security region code, security region name, and the municipality health service, and the total number of reported positive cases.

3.2.1.3. COVID-19 Hospital admissions

The number of patients admitted to hospitals for COVID-19 is a useful indicator to monitor the trends in SARS-CoV-2, though it depends on the structure of the health systems in different countries and most notably varied hospital admissions (García-Basteiro et al., 2020). Goethem et al. (2020), further explained that to assess the damaging effect of the SARS-CoV-2 infections in a population, monitoring of the hospital capacity through the COVID-19 hospital admissions is essential. They also explained that the COVID-19 hospital admission is an important indicator for the spatial and temporal spread of the SARS-CoV-2 on the national level. In the Netherlands, the government publicly provides open data of the daily COVID-19 hospital admissions supplied by the RIVM collected by NICE and LCPS (RIVM, 2020b). NICE and LCPS register and record the number of patients admitted into hospitals related to COVID-19. These numbers are made available to RIVM and published daily. The data explains the COVID-19 hospital admissions across different municipalities and safety regions in the Netherlands. The number of COVID-19 hospital admission data was downloaded from the source as a .csv file with columns such as daily hospital admissions, date of publication, the municipality name and code, security region code, security region name, and the municipality health service.

3.2.1.4. COVID-19 ICU admissions

The COVID-19 ICU admissions is another important indicator to monitor the dynamics of the SARS-CoV-2 by showing the virus transmission changes in an area over a certain period of time (García-Basteiro et al., 2020). Considering that COVID-19 hospital admissions is an effective measure of the severity of the SARS-CoV-2, the COVID-19 ICU admissions offer another means to understand the intensity of the virus in a population (ECDC, 2020). In the Netherlands, COVID-19 related ICU admissions are published based on the information provided by NICE and LCPS, which records the COVID-19 patients admitted into the ICU units of the hospitals. The data shows the COVID-19 ICU admissions across different municipalities and safety regions in the Netherlands. The number of COVID-19 ICU admission data was downloaded from the source as a .csv file with columns such as daily ICU admissions, date of publication, the municipality name and code, security region code, security region name, and the municipality health service.

3.2.1.5. COVID-19 related deaths

The number of COVID-19 related death is a significant SARS-CoV-2 epidemiological characteristic to explain the distribution of SARS-CoV-2 (Patel et al., 2020). Also, the SARS-CoV-2 is closely monitored by the WHO via a global monitoring system with different epidemiological indicators, including the COVID-19 deaths (WHO, 2021c). Also, in the Netherlands, the number of COVID-19 related deaths is one of the important indicators used in monitoring the spread of the virus within a population (RIVM, 2020b). The deaths due to COVID-19 are recorded by municipal health authorities, which the RIVM collects, reports, and publishes daily. The COVID-19 death data shows the number of COVID-19 deaths across different municipalities and safety regions in the Netherlands. The COVID-19 deaths data was downloaded from the data source as a .csv file with columns such as daily deaths, date of publication, the municipality name and code, security region code, security region name, and the municipality health service.

3.3. Data Preprocessing

3.3.1. Exploration of the SARS-CoV-2 wastewater data and the selected epidemiological indicators

Uncertainty may arise from the data collected or acquired from open sources due to data ambiguities, incompleteness, different attributes, and data inconsistencies, leading to the inability to generate trends from the dataset (Hariri et al., 2019). Therefore, considering the data used in this research are secondary data extracted from different data sources, aimed to describe the trends in the epidemiological indicators and their relationship, database management with PostgreSQL database using pgAdmin connector and R 4.0.4 statistical software have been identified as most suitable (Goethem et al., 2020; Viloria et al., 2019). The exploration of these indicators would help identify inconsistencies in the indicators, data types ambiguities and provides opportunities for a better understanding of the datasets for data preprocessing.

3.3.1.1. PostgreSQL data exploration

Data inconsistency in several indicators based on the data structure, data types and spatial attributes can be detected using PostgreSQL (Agarwal & Rajan, 2015). PostgreSQL is an open-source database management system for storing and investigating different attributes for consistency and completeness of the datasets to improve data usability (Stones & Matthew, 2005). Connection to the PostgreSQL database is possible through different means, including connection through the pgAdmin interface and by using psql located on the system's operating system (Stones & Matthew, 2006). The PgAdmin and psql interfaces offer the platform to ingest data into PostgreSQL, with the datasets in different file types such as .csv or json. Data in excel are sometimes unprocessed and have improper settings or appear in different

columns, but can be reorganized using the spreadsheet and separation of column attributes in excel for suitable analysis (Broman & Woo, 2018; Divisi et al., 2017). Therefore, upon acquiring the datasets of all the epidemiological indicators available as .csv container, the raw datasets combined the text or values into one cell, making the data difficult to read or ingest into the database. Hence, using excel, the combined text in the first column of the sheet separated by semicolon was split into different cells. The procedure provided a proper datasheet with separate columns for the contained attributes.

The organised excel format datasets of the selected epidemiological indicators and the SARS-CoV-2 wastewater RNA concentration data was ingested into the database. Firstly, a schema was created in the PostgreSQL database, where the datasets were stored. The pgAdmin interface was used to establish a connection to the database. The tables were created using .sql queries for the .csv files by specifying the table name, the columns, and the data types. The psql command line tool allowed ingestion of the .csv files into the database. To prevent errors during ingestion, the attributes of the tables were confirmed. The process was repeated for the individual .csv files, which create a table with the name and attributes of the SARS-CoV-2 wastewater RNA concentration data and the selected epidemiological indicators. Query operations were performed by filtering the WWTP name columns in the SARS-CoV-2 wastewater RNA data that corresponds with the municipality column in the positive cases data to identify the WWTP are present at the municipality level. Also, the query operation helps to ascertain the completeness of the SARS-CoV-2 wastewater RNA data corresponding to the other epidemiological indicators based on attributes such as dates and safety regions.

3.3.1.2. Spatial exploration of SARS-CoV-2 wastewater data

The exploration of the spatial attributes of datasets in a database is possible with the connection of the layer from the PostgreSQL database to a GIS environment such as QGIS (Sang et al., 2021). One of the significances of the PostgreSQL database systems is its capability of integrating GIS functionalities (Huisman & de By, 2009). Therefore, in order to explore the spatial attribute of the SARS-CoV-2 wastewater data, the wastewater treatment plant's visual inspection and understanding of its distribution spatially, as shown in figure 3-3, a QGIS connection was made to the database to display its location in space. Additionally, the municipality boundaries were overlaid on these locations for context to understand the distribution of these treatment plants within the municipalities. This gave an insight into the number of WWTP that fall within a municipality and room for consideration of the catchment areas of the WWTP. The WWTP system covers 322 municipalities, the percentage of contribution of the wastewater in these areas vary and determine the cumulative value per municipality. Therefore, the exploration further provides the need to consider the percentage of the wastewater flowing into the different WWTP, because the WWTP, in some cases, lack a one-to-one relationship with the municipalities. The WWTPs are distributed such that one municipality has one or more WWTPs. This created the need to convert the SARS-CoV-2 wastewater readings from WWTP to municipality. Similarly, the inspection of the other tables containing the positive cases, hospital admissions and deaths show a daily record for the 352 municipalities in the Netherlands as identified in the PostgreSQL relational database.
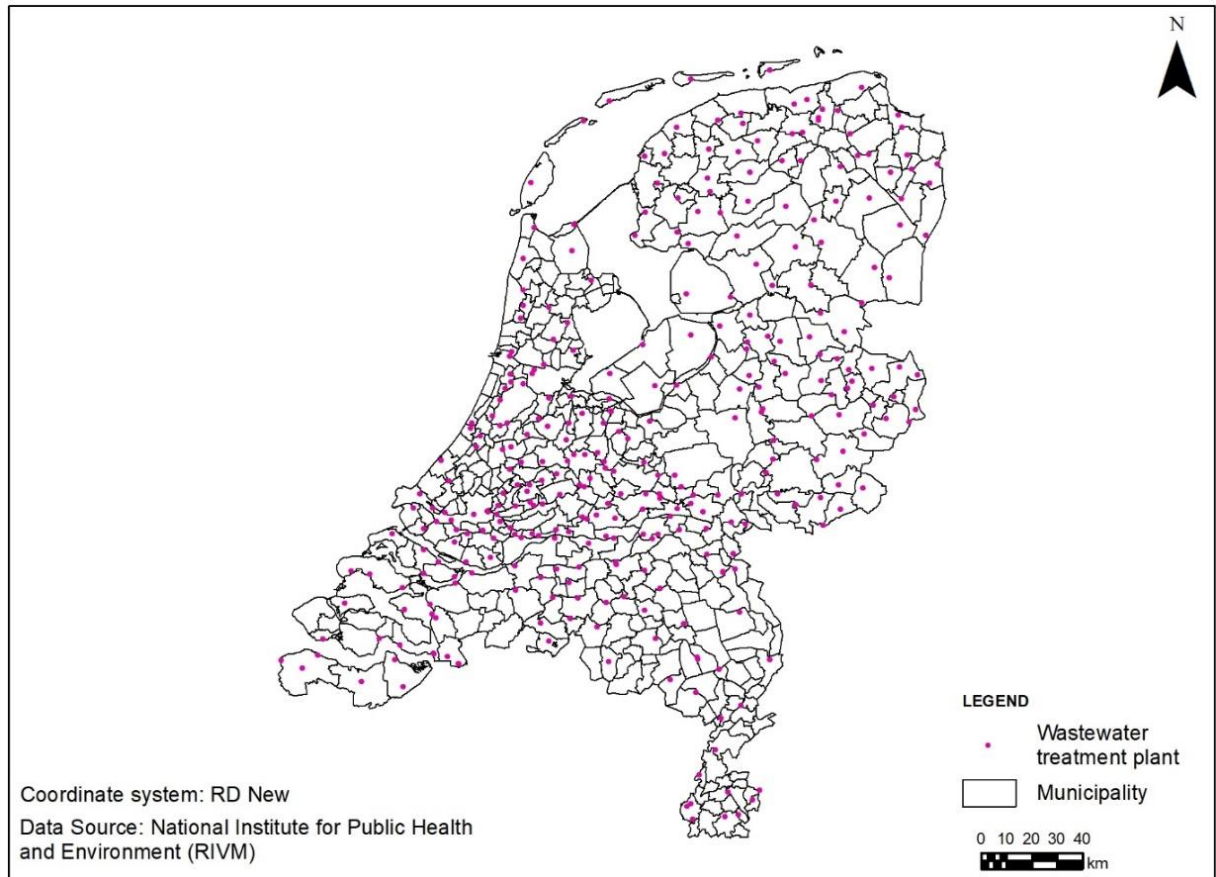
Figure 3-3: The 322 Wastewater treatment plants (WWTP) in the Netherlands. Own design, data from RIVM (2020d)

3.3.2. Organisation of the SARS-CoV-2 wastewater data and the selected epidemiological indicators

The SARS-CoV-2 wastewater data and the epidemiological indicators were initially only available in different forms aggregated at weekly and daily scales, respectively. They needed to be organized to a uniform temporal and spatial unit of analysis in order to compare the WBE data with other epidemiological indicator data (Escolà Casas et al., 2021; González-Mariño et al., 2020; Goulding et al., 2020; Thomas et al., 2012). According to Escolà Casas et al. (2021), a uniform and fine spatial resolution should be selected as a common unit of analysis to efficiently describe the potential of WBE for spatial and temporal analysis. Therefore, in this thesis, the epidemiological indicators and the SARS-CoV-2 wastewater data are organized to the municipality level as the elementary unit of analysis common to the selected indicators (Escolà Casas et al., 2021).

The conceptualization of the processes for a common unit of analysis provides a detailed, clear pathway for the organization of the datasets. The municipality is defined as the common unit of analysis for the datasets. The process and steps involved in bringing the datasets to a common unit of analysis are shown in Figure 3-4. The figure also outlines the procedures of organizing all the epidemiological indicators, including the SARS-CoV-2 wastewater RNA concentration data, to be suitable for spatial and temporal analysis. This concludes the steps for the first research objectives to organise all the epidemiological indicators for a suitable spatial and temporal analysis.
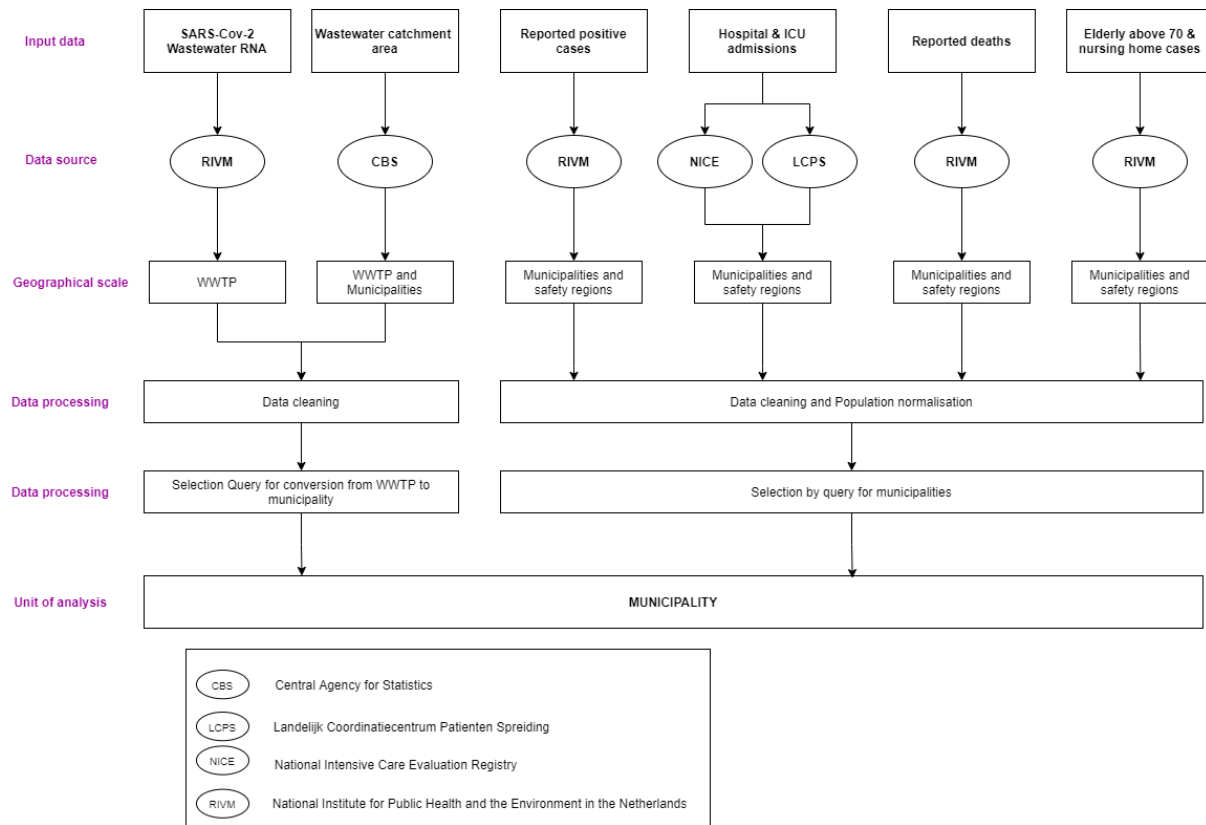
Figure 3-4: Steps for datasets modification for a common unit of analysis, data management and organisation

Upon download of the research data, following the data acquisition and data management procedures as explained in sections 3.3.1, since most of these indicators exist in an unstructured format, R statistical software was used to clean and organise the epidemiological indicators used in this research. Harmonisation of epidemiological indicators through cleaning and managing these indicators is an important step in epidemiological studies since most of these indicators exist in an unstructured format (Owada et al., 2016). The cleaning of the datasets commenced with transforming the data type of the dates column from factor to date format, which should be the basic format of the column (Jonge & Loo, 2013). The date format is an essential and common column for all the indicators to organise the datasets together. Also, Jonge and Loo (2013) highlighted removing duplicate rows or rows with missing values as an important feature of data cleaning. Hence, the reading of the selected epidemiological indicators such as reported cases, hospital admissions and reported deaths, ICU admission cases, nursing home cases and cases of people above the age of 70 that are not assigned to any municipality were removed.

Furthermore, inappropriately assigned special characters such as commas, asterisks or full stops were removed, and rows with such misappropriation were corrected. For example, a column with numeric values with commas was corrected, and the names of the municipalities were checked for both population data and the epidemiological indicator data, while those names that are inconsistent but have the same codes are corrected. Furthermore, data cleaning was used to explore the municipalities that exist in the wastewater data and the other epidemiological data, confirming one or more WWTP exist within a municipality.

Population normalization of epidemiological indicators

Population normalization is an important consideration in the application of wastewater-based epidemiology, useful for estimating the population contributing to the samples of the wastewater collected from the WWTP (Polo et al., 2020). It is also essential for comparison between wastewater virus concentration signals and other epidemiological indicators. In the Netherlands, RIVM has considered the population normalization for the SARS-CoV-2 wastewater data of the WWTP from September 2020. The data was collected in the form of the virus RNA value per 100,000 inhabitants. Therefore, in this study, the population normalization of the other epidemiological indicators was computed to enable proper comparison with the SARS-CoV-2 wastewater data. The population normalization of the selected epidemiological indicators (section 3.2.1) was calculated using the reading of the epidemiological indicators and the number of inhabitants per municipality collected from CBS statistics (CBS, 2020b). The formula for the population normalization for the epidemiological indicators per 100.000 inhabitants is as shown in equation 2. The formula was applied to all the selected epidemiological data across the municipalities.

$$Normalised\ cases\ = \left(\frac{number\ of\ cases}{number\ of\ inhabitants}\right) * 100000 \dots\dots\dots\dots\dots\dots\dots\ (2)$$

The concluding organization of the epidemiological indicators involves converting the daily reading of the epidemiological indicators to a weekly sum for adequate comparison with the weekly reading of the SARS-CoV-2 wastewater data. Also, the conversion of the date column in the data of all the epidemiological indicators to a uniform date. The readings of the epidemiological indicators were then summarized per week. The query selection process involves the calculation of the weekly sum of readings for the population normalized epidemiological indicators.

Conversion of wastewater reading from WWTP to municipality

For the selection query for the conversion of the SARS-CoV-2 wastewater data from WWTP to municipality level, the unit of analysis was considered. Firstly, The SARS-CoV-2 wastewater data comprises reading recorded weekly readings of the RNA concentration value but was recorded on different dates within the week. For example, a treatment plant records on day one while another records on day two, their dates are different but within the same week. So, there is a need to have a uniform date for readings obtained within the week different treatment plants. The week dates were first converted into week numbers and then each week number was assigned the first day of the week. The file containing the catchment area of the WWTP which includes the percentage wastewater contribution from the different municipalities into the WWTP and the cleaned file containing weekly reading of the SARS-CoV-2 wastewater RNA concentrations, were used in the selection query to organize SARS-CoV-2 wastewater RNA reading from WWTP scale to municipality scale. The query operation worked on the file containing the readings per WWTP and the file containing the municipality percentage contribution to WWTP and check the WWTP codes in both files and merges them based on the WWTP codes. With the consideration of the dates, the weekly reading for the WWTP was then queried based on the percentage of the municipalities to obtain the weekly reading of the WWTPs in a municipality. Then, the average of the weekly reading that falls within a municipality for the week was computed. The data was then transformed such that the municipalities are rows while the weekly readings are restructured in a column that follows the date for the readings, and the municipality names was then joined to the data file.

In this thesis, the analysis was performed at three geographical scales, the national scale, safety region and the municipality level. The organization of all selected epidemiological indicators to the national level involve a query operation that returns the weekly sum of the readings across all the municipalities. The file for the SARS-CoV-wastewater data was then merged with the selected epidemiological datasets based on the date. Hence, an organized weekly reading of all the epidemiological indicators at the national scale was generated. The weekly reading for these SARS-CoV-2 wastewater data was validated by selecting 10 random municipalities in the new data file and by manually computing the weekly values based on the wastewater percentage contribution from the municipalities to the WWTP using Microsoft Excel.

The code developed for the organization of the SARS-CoV-2 wastewater data and the selected epidemiological data and generation of spatio-temporal trends is published on GitHub at (https://bit.ly/3jZMWtl). GitHub is a platform that remotely stores code versions and implementation.

3.4. Spatial and temporal analysis of the epidemiological indicators

3.4.1. Temporal exploration

The temporal exploration of epidemiological indicators of an infectious disease such as COVID-19 is an important consideration to understand the patterns and trends in the relationship between these indicators. In order to explore the relationship between HIV/AIDS incidence and deaths per 100,00 inhabitants in China between 2004 and 2017, Xu et al. (2020) used R-statistical software to generate the trends and the relationship between the epidemiological indicators on a national scale. Saguti et al. (2021) also used R to produce trends in the SARS-CoV-2 wastewater concentration and a number of hospitalised COVID-19 cases in Gothenburg, Sweden. The result explained the trends reveal the pattern of the datasets with visual identification of the peaks in the datasets and how they relate in time. Following these examples, in this study, the R- statistical software was used to produce the trends in the SARS-CoV-2 wastewater RNA concentration and individual epidemiological indicators on the national scale. Using the file containing normalised weekly reading of all the epidemiological indicators, the trends in SARS-CoV-2 RNA concentration in wastewater over the study period and the selected epidemiological indicators were produced.

The visual overview of the temporal exploration of the normalised SARS-CoV-2 wastewater RNA and the other epidemiological indicators shows some descriptions of the temporal patterns in these datasets, hence further explaining the strong and weak relationship (D'Aoust et al., 2021). To explore the potential of the SAR-CoV-2 wastewater data as a measure of the spatial and temporal analysis of the virus, the epidemiological indicator with a strong relationship is needed. The visual overview of these indicators shows the epidemiological indicators with a strong relationship with the SARS-CoV-2 wastewater RNA signal. Based on this, the COVID-19 positive confirmed cases, hospital admissions and COVID-19 deaths were selected for further analysis.

Since the epidemiological indicators were organised to the municipality and safety regions level, there is a need to observe the trends in these epidemiological indicators to reveal their patterns across these levels. R-shiny is an important tool in exploring generating trends and exploration of epidemiological indicators across multiple scales (Tebé et al., 2020), which was used to generate the temporal trends in the SARS-CoV-2 wastewater concentration and the epidemiological indicators across the municipalities and safety regions in the Netherlands. However, there is a need to explore the spatial patterns of the time series of the epidemiological indicators and the SARS-CoV-2 wastewater concentration to describe the potential of the SARS-CoV-2 wastewater data to describe the transmission of the virus.

3.4.2. Time series mapping

The spatial analysis of the COVID-19 epidemiological indicators reveals the spatial patterns across different geographical units (Zhang & Schwartz, 2020). Different studies explored the spatio-temporal patterns of COVID-19 positive cases across different geographical scales and is a good measure of the SARS-CoV-2 transmissions (Kang et al., 2020). Therefore, the COVID-19 positive cases is a suitable epidemiological indicator to compare with the SARS-CoV-2 wastewater RNA concentration to explore the spatio-temporal patterns of the SARS-CoV-2 transmissions. In comparing two epidemiological indicators spatially, an adequate method is a time series map across different spatial units produced using ArcGIS (Zuo et al., 2020). For this reason, time-series maps of the SARS-CoV-2 wastewater data and the positive confirmed cases were generated using ArcGIS in this research. The study by Blanford and Jolly (2021) shows two weeks summary of time-series maps of COVID-19 weekly positive cases in the Netherland. Time-series maps were considered as reference for the time series maps generated in this study, and the second week in each month from September 2020 till June 2021 was considered for the spatial time series. Using ArcGIS, the epidemiological data was joined to the municipality shapefile, and the weekly values of SARS-CoV-2 wastewater RNA concentration and positive cases were mapped (Figures 4-3 and 4-4). Similarly, to generate the time-series maps across the safety regions in the Netherlands, a .csv file containing the safety region names, safety region codes and their corresponding municipality code. The .csv was joined to the municipality shapefile, then the municipality within the safety region was dissolved to obtain the shapefile showing the safety region. The process of generating the municipalities time series maps was applied across the safety regions in the Netherlands (Figures 4-5 and 4-6).

3.5. Statistical association between the epidemiological indicators

In describing the statistical association between the time series of normalised SARS-CoV-2 wastewater RNA and the selected epidemiological indicators, bivariate Pearson Correlation analysis was used and implemented in SPSS. The bivariate Pearson Correlation involves the analysis of pairs of indicators and assessing the significance of the relationship between these indicators. In this research, the statistical association between the pairs of the time series of normalised SARS-CoV-2 wastewater RNA and Covid-19 positive cases, hospital admissions and reported deaths were calculated across the municipality, safety region and national scales. The bivariate Pearson Correlation analysis generates a Pearson Correlation coefficient, r, for the relationship between these indicators across the geographic unit of analysis. Upon generation of the Pearson correlation coefficients, the spatial analysis of the coefficients was mapped across safety regions (Figure 4-6) and municipalities (Figure 4-7) using ArcGIS. The spatial analysis of the bivariate Pearson correlation results enables the visualisation of the spatial patterns of these correlations across the different geographic scales (Figures 4-6 and 4-7).

3.6. Temporal association between the epidemiological indicators

In understanding the relationship between two different time series, the cross-correlation function (CCF) is a method the examines the temporal cross-correlation or cross-covariance of the time series. Regression models such as ARIMA model are used for multivariate analysis, which involves the temporal relationship between several variables. Granger causality test deals with the causal relationship between trends of two variables and tests the usefulness of one time series to predict the other. The cross-correlation function is useful in identifying the time shift in two different time series (Souza & Felix, 2018). Considering the data of the indicators in this study, CCF is suitable to determine the variable that precedes another and the statistical significance of correlation between the variables. The temporal relationship between the time

series of SARS-CoV-2 wastewater concentration and positive cases within the period (September 2020 till June 2021) was considered. Therefore, the CCF was employed to describe the time shift between the time series of weekly readings of normalised SARS-CoV-2 wastewater RNA concentration and the positive cases at the municipal level using the SPSS software. The time shifts of the CCF are produced in the form of time graphs that describe the relationship between the time series of these indicators. To examine the CCF between the normalised SARS-CoV-2 wastewater RNA and reported positive cases, the SARS-CoV-2 wastewater RNA is referred as the y variable and the positive cases as the x variable. Here, a negative CCF time shift would mean the SARS-CoV-2 wastewater RNA concentration predates the positive cases by the identified lag value of the CCF time plot (Appendix 1).

The analysis commenced by defining the sample covariance function as

$$g_k^{xy} = \frac{1}{n}\sum_{t=1}^{n-k}(y_t - y)(x_{t+k} - x) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3)}$$

Then, the relationship between the variable y was estimated and at different time-shifted variable x_{t+k}. Also, the 95% confidence interval on the CCF was estimated using:

$$-\frac{1}{n} \pm \frac{2}{n} \quad\quad\quad\quad \ldots\ldots\ldots\ldots\ldots\ldots..\ldots. \text{(4)}$$

Where n is the number of observations used in the calculation of the cross-correlation function.

The confidence interval was calculated with (n = 19) is as shown on the CCF plot as using the equation (4).

In the CCF plot, the time shift of the highest cross correlation value between these indicators for individual municipality was recorded in a data sheet saved as .csv. The significance of the CCF plot was also observed and recorded, the trend with no significance is recorded as 0, and the trend with significance is recorded as 1. The CCF value is also generated using the SPSS software describing the correlation between trends in SARS-CoV-2 wastewater data and the positive cases at the different time lag (-2 to 2) set for the CCF model. The highest and the second highest CCF value were recorded (Appendix 2)

The spatial analysis of the time shift between the SARS-CoV-2 wastewater RNA and positive cases provides a clear understanding of the spatial patterns of the temporal relationship between the epidemiological indicators. Therefore, ArcGIS was used to generate the map displaying spatial patterns of the time shift (Figure 4-8) and cross-correlation value (Appendix 3B) at municipality level. The output of the CCF and the spatial analysis of the time shift explains the potential of the SARS-CoV-2 wastewater data as an early warning indicator to describe the SARS-CoV-2 transmission intensity across the municipalities in the Netherlands.

3.7.   Ethical considerations

Studies addressing health-related issues must guarantee that the research protocols, goals and strategy protect the wellbeing of individuals concerned. Therefore, ethical considerations are important when dealing with health-related data. This research used secondary health data, which means that the data has been collected and existed on an officially published government platform, is an open-source dataset collected and published by the RIVM and does not contain individual, personal or private information of the infected individuals, and no identifiable variable is used. Also, the RIVM had considered all privacy issues by aggregating to the municipality level, hence the use of these data does not pose any ethical concerns. The minimum geographical scale the research findings are available is the municipality level and would complement the understanding of the health-risk of SARS-CoV-2. The result of this research will be communicated to the RIVM and be placed under the authority of ITC and RIVM and would be available upon request.

4.   RESULTS

This chapter describes the relation between SARS-CoV-2 wastewater RNA concentration and selected epidemiological indicators across space and time (section 4.1), identifies the statistical association between SARS-CoV-2 wastewater RNA concentration and selected epidemiological indicators (section 4.2) and analyses in whether and how far SARS-CoV-2 wastewater RNA concentration can be used as an early warning indicator for transmission of SARS-CoV-2 (section 4.3).

4.1.   Spatial and temporal analysis of SARS-CoV-2 wastewater RNA and other epdemiological metrics

The relationship between the SARS-CoV-2 wastewater RNA and the selected epidemiological indicators are presented in two sections. Firstly, the pairwise temporal trends result of the normalized SARS-CoV-2 wastewater RNA concentration and the selected four epidemiological indicators is presented. Secondly, the spatio-temporal analysis of the SARS-CoV-2 wastewater RNA concentration and the reported positive cases at safety region and municipality level is presented.

4.1.1.   Temporal trends in the SARS-CoV-2 wastewater RNA and the selected epidemiological indicators

The weekly variation of the normalised SARS-CoV-2 wastewater RNA concentration and the four epidemiological indicators is shown in Figure 4-1. A visual comparison of the trends in SARS-CoV-2 wastewater RNA concentration and the reported positive cases (Figure 4-1A) indicates that the wastewater RNA signals show a more or less similar pattern with the reported positive cases. In most instances, a considerable rise and fall in the wastewater RNA signal is similar to the positive cases trends. For example, similar a synchronous peak in both wastewater signal and reported positive cases on the week of the 26th of October 2020 (Label 1), 21st of December 2020 (Label 3), 22nd March 2021 (Label 6), and 3rd May 2021 (Label 7). There is also a steady decline of both trends, the wastewater RNA concentration and the positive cases as shown in Label 7. An alternating pattern can be observed as indicated in Labels 2, 4 where a peak in wastewater RNA concentration corresponds with a decline in positive cases. In short, a partly similar pattern can be identified between the wastewater RNA signal and the positive cases signal during the study period.

The visual comparison of the trends in SARS-CoV-2 wastewater RNA concentration and the hospital admissions (Figure 4-1B) also shows a partially similar, but not consistent temporal trends of both indicators. The similarities can be observed with parallel patterns of rise and fall in the two trends as indicated from 22nd of March 2021 until 14th of June 2021 (Label III). The comparison of the trends in SARS-CoV-2 wastewater RNA concentration and the hospital admissions show some inconsistent patterns, with alternating rise and fall of the signals. A peak and decline in wastewater signal have a corresponding rise and fall in hospital admission signal after about one or two weeks (Label I and II). Hence, the wastewater RNA signal has similar patterns as the trends in COVID-19 related hospital admissions, but some inconsistencies were observed.

The comparison of the wastewater RNA signal and the trends in ICU admissions (Figure 3-1C), and reported deaths (Figure 3-1D), show few parallel patterns, and some inconsistencies in the trends can be observed. Figure 3-1C reveals a similar pattern in wastewater RNA signal and the trends of ICU admissions at most times during the study period, but the ICU admissions trend does not portray a similar distinct rise and decline as observed in wastewater RNA signal. Figure 3-1D illustrates a related pattern in the wastewater RNA signal and the trends in COVID-19 reported deaths. The rise and drop in COVID-19 reported deaths appears weeks after the corresponding rise and drop in the wastewater signal (Label I).

Label II in Figure 3-1D indicates a peak in wastewater RNA concentration corresponds with a decline in COVID-19 related deaths. Therefore, the decline in COVID-19 related deaths and the inconsistency with the RNA concentration might be due to some factors such as the effect of vaccination, thereby reducing the COVID-19 related deaths.

Generally, there are similarities and important differences in the visual exploration of the trends in SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators. The visual comparison is insufficient to describe the relationship between the trends in SARS-CoV-2 wastewater RNA concentration and epidemiological. The visual exploration will be complemented with a statistical analysis. The SARS-CoV-2 wastewater RNA signal largely shows similarities with the COVID-19 reported positive cases signals during the study period. Hence, the further exploration of the relationship between SARS-CoV-2 wastewater RNA concentration and positive cases across smaller geographical scales such as the safety region and municipality.
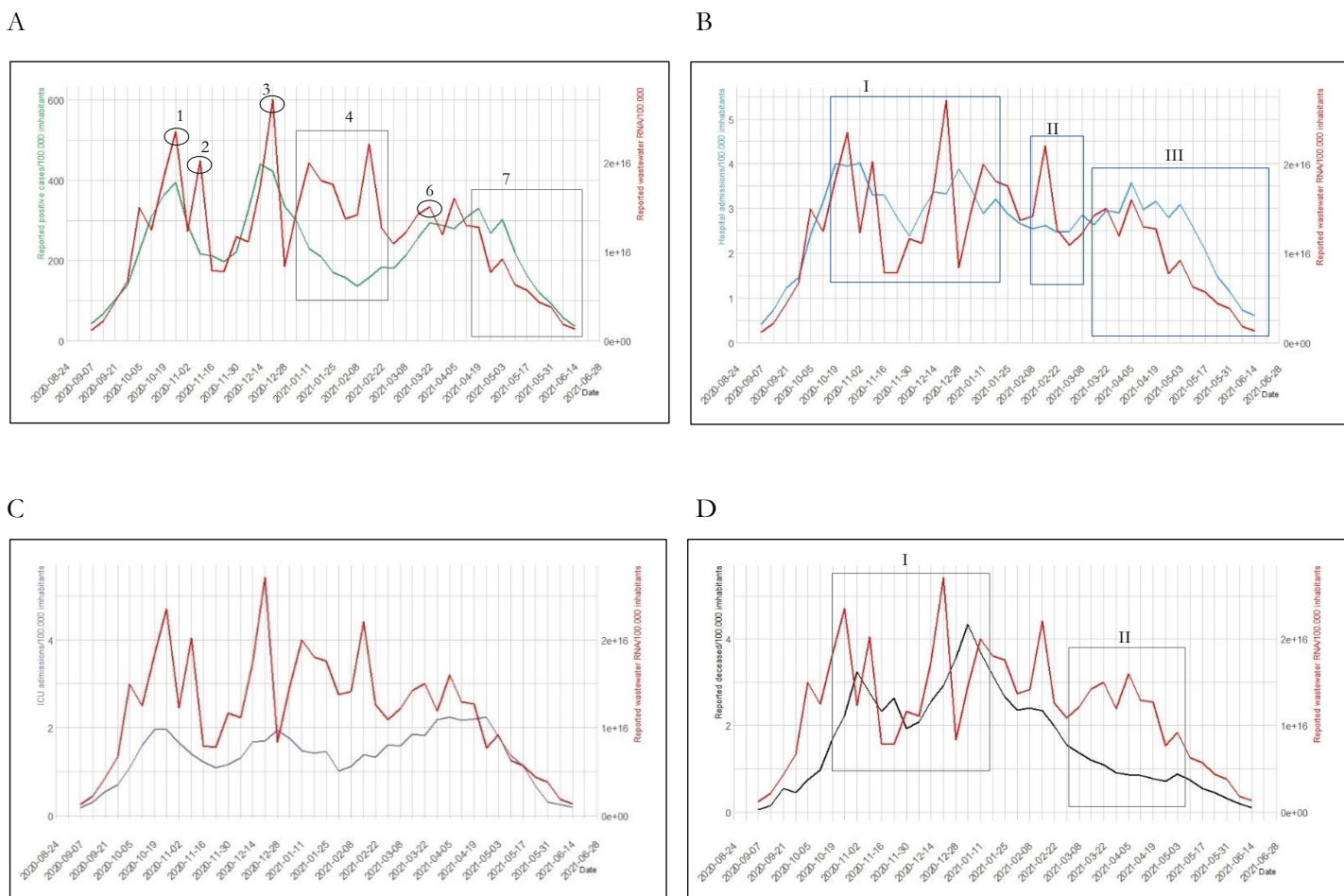

Figure 4-1: Epidemiological metrics of normalised SARS-CoV-2 RNA concentration in wastewater and (A) reported positive cases, (B) hospital admissions, (C) ICU admissions, (D) reported deaths, per 100.000 inhabitants at the national level over the study period

4.1.2.   Spatio-temporal analysis of the SARS-CoV-2 wastewater RNA and reported positive cases

In this section, we zoom in from the national to the safety region and municipality level. The spatio-temporal visualisation and analysis of the epidemiological indicators reveal that substantive variation occurs at the regional and municipal level. The time-series maps of the SARS-CoV-2 wastewater data and the reported positive cases show the virus transmission intensity across safety regions (Figure 4-2 and 4-3) and municipalities (Figure 4-4 and 4-5).

Figures 4-2 and 4-3 provide a comparative temporal overview of the wastewater RNA concentration and reported positive cases across safety regions in the Netherlands. The comparison of the wastewater RNA concentration with the positive cases, send a mixed message. On one hand, similar spatial patterns can be observed in both indicators. On the other hand, there are inconsistent spatial patterns across the safety regions at different times.

Figure 4-4 and 4-5 provides a similar comparative overview of the wastewater RNA concentration and reported positive cases, but now at the more detailed municipality level, with similar patterns of increase and decrease in the virus intensity in both indicators during the study period. Comparing the time series of SARS-CoV-2 wastewater data and positive cases in these figures, the virus intensity variations were observed but not consistent across the study period.

Generally, the disproportional distribution of SARS-CoV-2 wastewater RNA concentration and reported positive cases reveals the safety regions and municipalities with high rates of incidences through the epidemiological indicators during the study periods. Considerable similar patterns can be observed in the wastewater data and the positive cases across the safety regions than the municipality. Based on these observations, the wastewater RNA concentration data can describe the spatial and temporal patterns of SARS-CoV-2 transmission in the Netherlands. There are clear similarities and clear differences in the SARS-CoV-2 virus intensity variation across safety region and municipality based on the comparison of the spatio-temporal trends in the wastewater RNA concentration and the reported positive cases. The time series maps reveal similar and inconsistent patterns in areas with very low and high virus intensity at the safety region and municipality level. The revelation of the similarities and differences from the visual exploration of the spatio-temporal trends make it hard to draw firm conclusions on the relationship between the SARS-CoV-2 wastewater RNA concentration and the positive cases. Therefore, a statistical analysis is needed to complement the visual exploration of the SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators.

Figure 4-2: Spatio-temporal trends of SARS-CoV-2 wastewater RNA concentration and reported positive cases across safety regions in the Netherlands from September 2020 to January 2021

Figure 4-3: Spatio-temporal analysis of SARS-CoV-2 wastewater RNA concentration and reported positive cases across safety regions in the Netherlands from February 2021 to June 2021

Figure 4-4: Spatio-temporal analysis of SARS-CoV-2 wastewater RNA concentration and reported positive cases across municipalities in the Netherlands from September 2020 to January 2021.
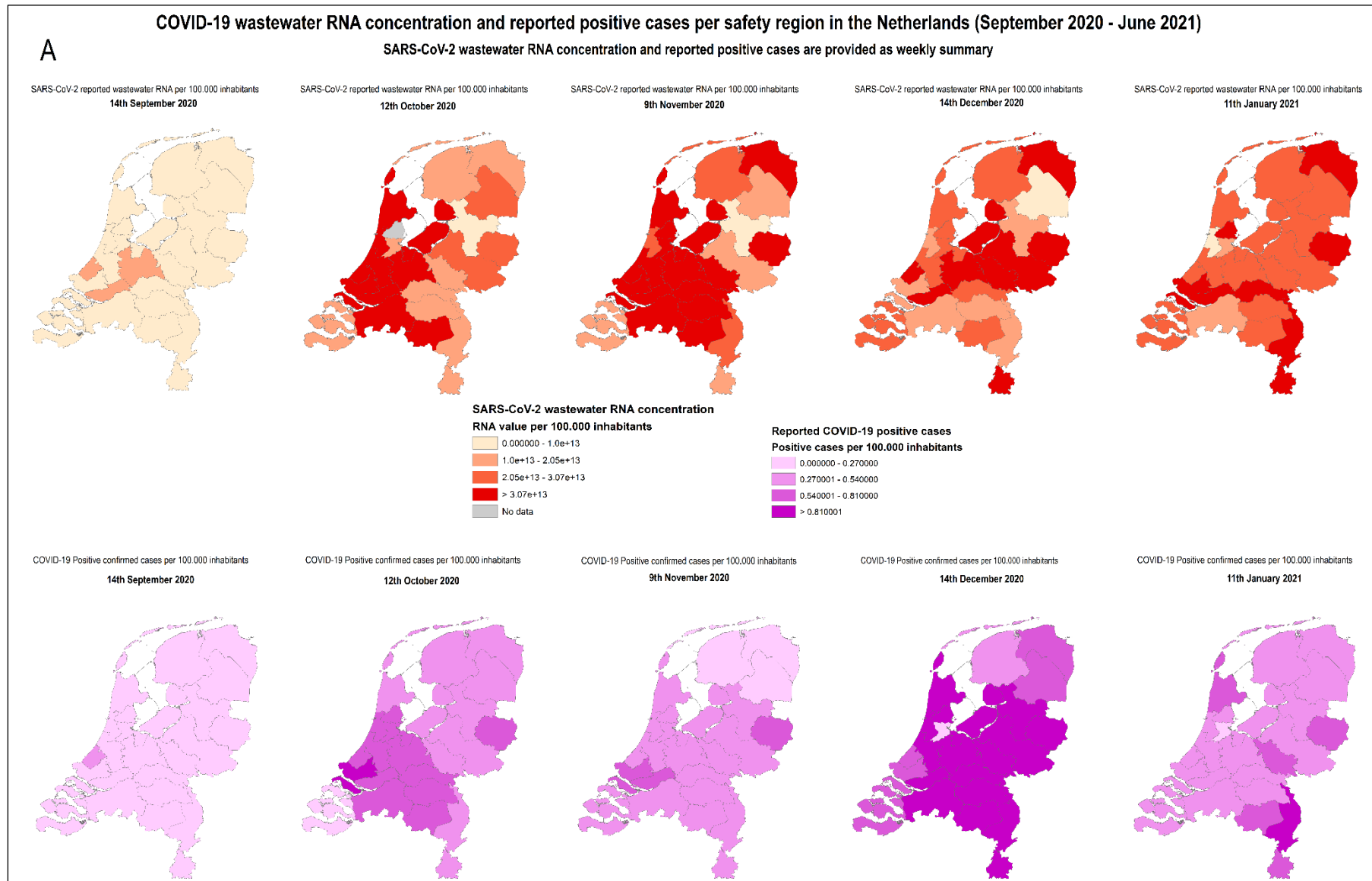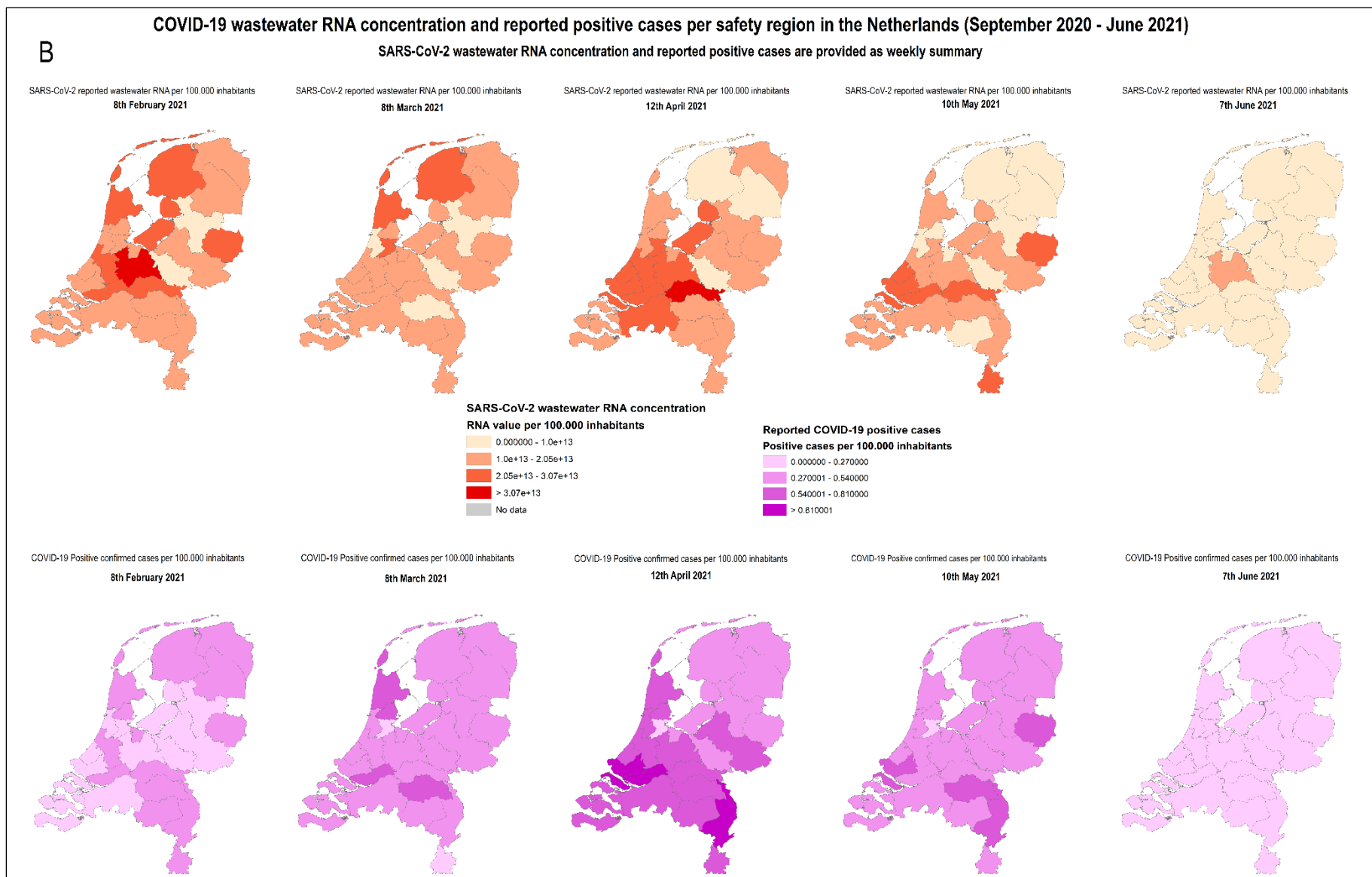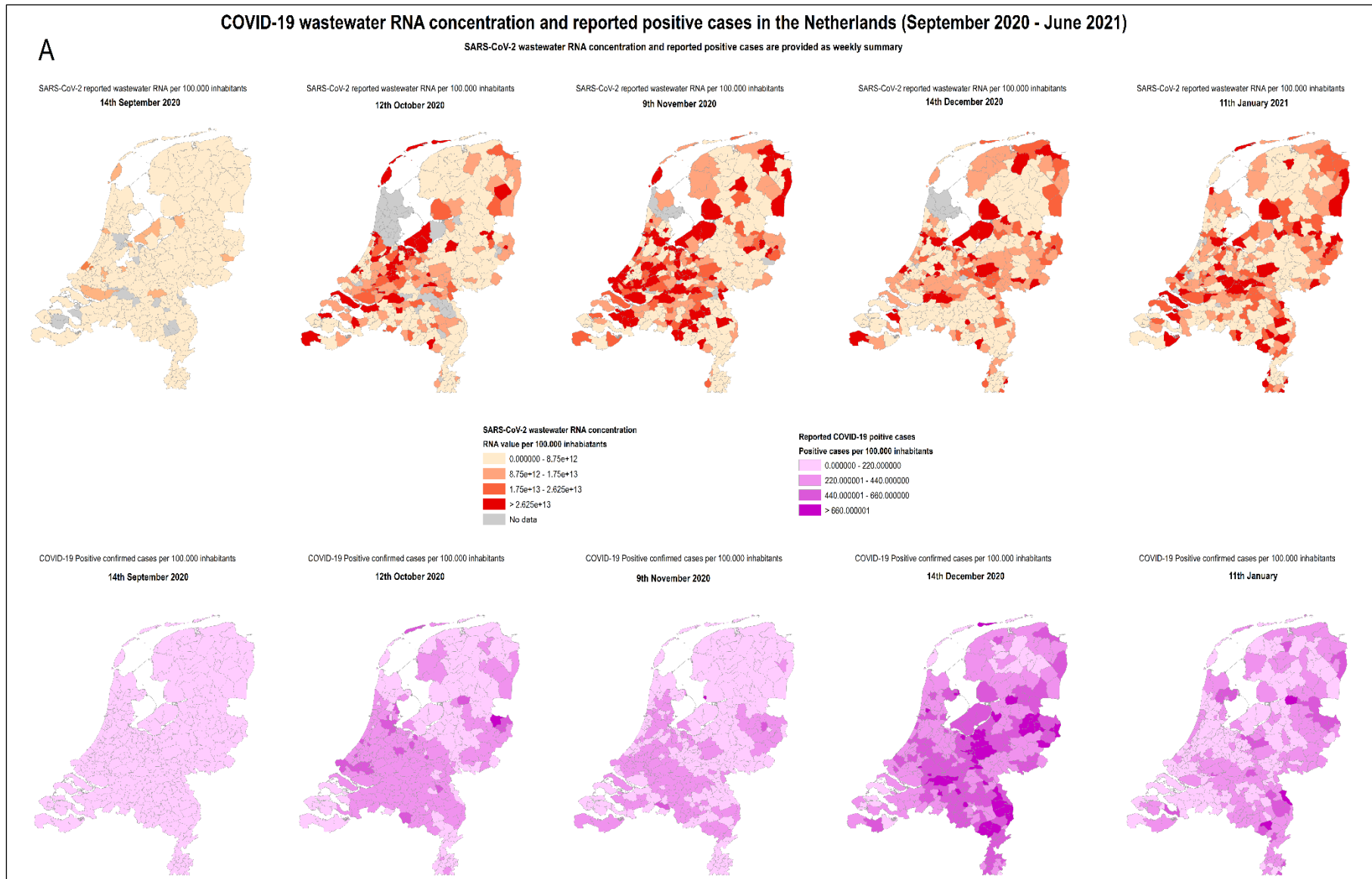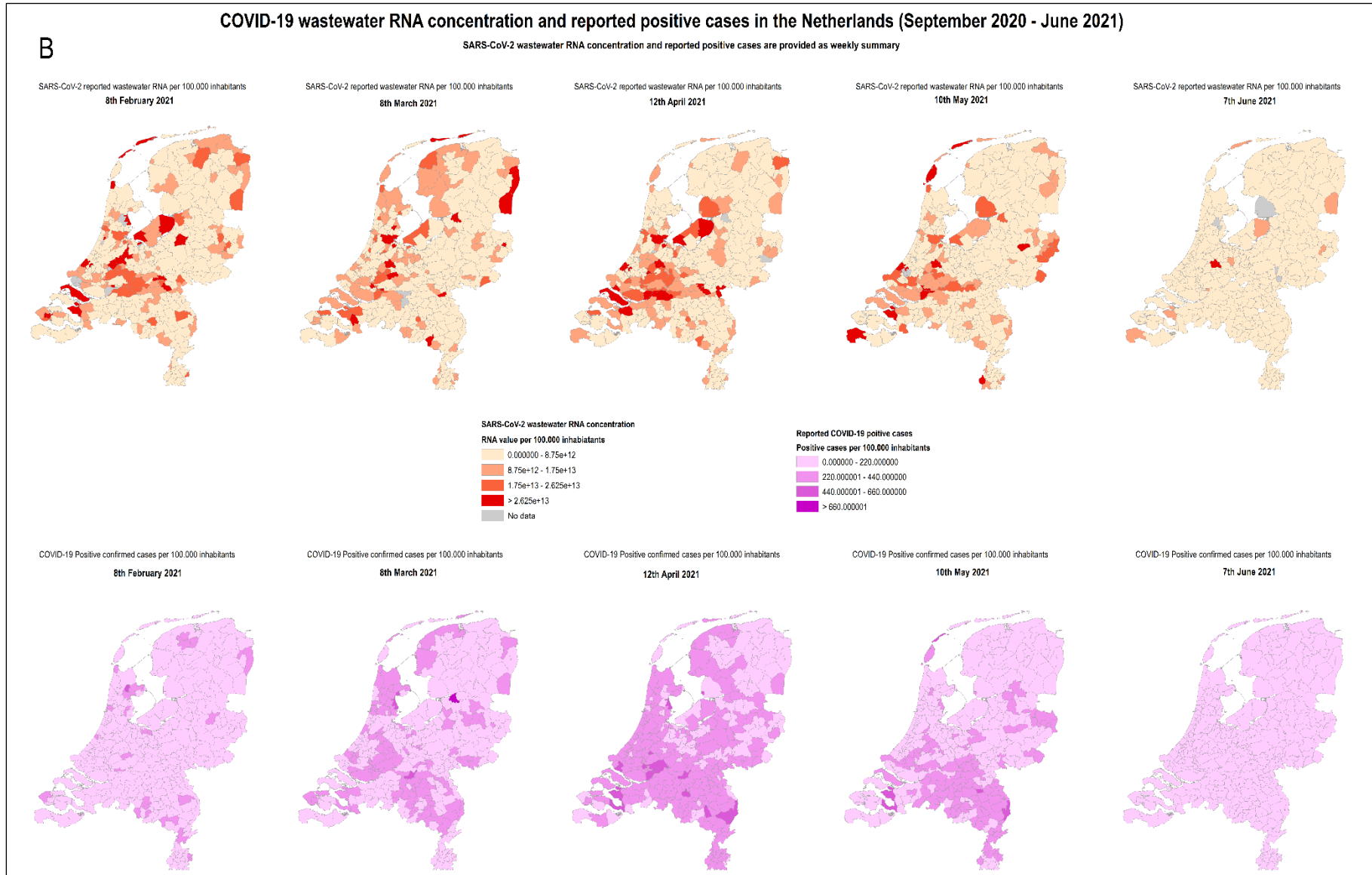
Figure 4-5: Spatio-temporal analysis of SARS-CoV-2 wastewater RNA concentration and reported positive cases across municipalities in the Netherlands from February 2021 to June 2021

4.2. Statistical association between SARS-CoV-2 wastewater RNA concentration and selected epidemiological indicators

The association between the trends SARS-CoV-2 wastewater RNA concentration and the epidemiological indicators was examined by means of a bivariate Pearson correlation analysis after which results were visualized at three geographical scales, national, safety region and municipality. Three out of the four epidemiological indicators were chosen: reported positive cases, hospital admissions and reported deaths. As the ICU admission data are only available on the national scale, so they were excluded from the analysis presented. The statistical association between the trends in the SARS-CoV-2 wastewater RNA concentration and the three selected indicators are presented in the following subsections.

4.2.1. Statistical association between SARS-CoV-2 wastewater RNA concentrations and selected epidemiological indicators at the national scale

The results of the bivariate Pearson correlation at the national scale, is presented in Table 6. The null hypothesis states that:

'There is no significant relationship between the SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators.'

The Pearson correlation coefficient indicates a strong positive correlation between the SARS-CoV-2 wastewater RNA concentration with each of the epidemiological indicators. Specifically, the SARS-CoV-2 wastewater RNA concentration has a strong positive correlation with hospital admissions ($r = 0.727$, $p < 0.01$) and reported positive cases ($r = 0.655$, $p < 0.01$) and reported death ($r = 0.634$, $p < 0.01$). The positive correlation coefficient clearly indicates that a good match exists between the trends in wastewater RNA concentration and the selected epidemiological indicators. The result also indicates that the relationship between the SARS-CoV-2 wastewater RNA concentration with each of the epidemiological indicators is statistically significant, with the p-value less than 0.01 within the confidence interval. Hence, we reject the null hypothesis. A clear relationship between the SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators has been identified at the national level, hence, the need to further explore the statistical association between these epidemiological indicators at the subnational level (safety region and municipality).

Table 6: Bivariate Pearson correlation for SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators at the national level

Correlations		RNA concentration	Reported positive	Hospital admissions	Reported deaths
RNA concentration	Pearson Correlation	1	.655**	.727**	.634**
	Sig. (2-tailed)		.000	.000	.000
	N	41	41	41	41
**. Correlation is significant at the 0.01 level (2-tailed).					

4.2.2. Statistical association between SARS-CoV-2 wastewater RNA concentrations and selected epidemiological indicators at safety region and municipality level

The spatial variation of the bivariate Pearson correlation between the SARS-CoV-2 wastewater RNA and the three epidemiological indicators is presented across the safety regions in Figure 4-6 and municipality in Figure 4-7. The statistical significance is at 95% confidence interval. The variation of the correlation coefficients across the safety region (see Figure 4-6A) shows that the relationship between the SARS-CoV-2 wastewater RNA and reported positive cases are statistically significant in nearly all safety regions. Eight safety regions (see Figure 4-6A) have a strong positive correlation coefficient between 0.6 and 0.8. Many safety regions have a positive correlation between 0.4 and 0.6, and only one safety region is statistically insignificant. The statistical association between the SARS-CoV-2 wastewater RNA and the hospital admissions (see Figure 4-6B) shows some safety regions are statistically insignificant, about five safety regions have strong positive correlation with Pearson correlation value between 0.6 and 0.8, and some safety region with weak positive correlation between 0.2 and 0.8. The statistical association between the SARS-CoV-2 wastewater RNA concentration and reported deaths (Figure 4-6C) indicates nine safety regions are statistically insignificant, nine safety regions with a positive correlation coefficient between 0.4 and 0.6 and the rest with a weak positive correlation between 0.2 and 0.4.

Generally, the SARS-CoV-2 wastewater RNA is more statistically significant and has a stronger positive correlation with the positive cases across the safety region than most of the hospital admissions and the reported deaths.

Figure 4-6: Correlation coefficient per safety region between SARS-CoV-2 wastewater RNA concentration and (A) reported positive cases, (B) hospital admissions and (C) reported deaths per 100.000 inhabitant the Netherlands from September 2020 to June 2021.

Pearson correlation between SARS-CoV-2 wastewater RNA and positive reported cases, hospital admissions and reported deaths at municipality level is presented in Figure 4-7. The statistical significance of the correlation was computed at 95% confidence interval. Figure 4-7A indicates that the relationship between the SARS-CoV-2 wastewater RNA and reported positive cases are statistically significant with medium to strong positive correlation for many municipalities in the Netherlands. It however also shows that there is no significant relation for 109 (30.9%) municipalities. Figures 4-7B and 4-7C reveal that the SARS-CoV-2 wastewater RNA generally does not have a statistically significant relation with hospital admissions and reported deaths for 280 (79.5%) and 287 (81.5%) municipalities respectively. Generally, the spatial variation of the correlation between SARS-CoV-2 wastewater RNA concentration and three epidemiological indicators is obvious across municipalities and safety regions than on the national level. The obvious correlation between the epidemiological indicators across safety regions and municipalities describes the heterogeneity of the significance of SARS-CoV-2 virus transmission across the regions and municipality. The SARS-CoV-2 transmission occurs at a different speed in different localities which act as the scale of measurement of infection rate nationally; hence the municipality is the spatial area of interest for further analysis. The result indicates that the positive cases are the most significant and most positively correlated epidemiological indicator with the SARS-CoV-2 wastewater RNA concentration. Hence, the selection of the positive cases for further temporal exploration.
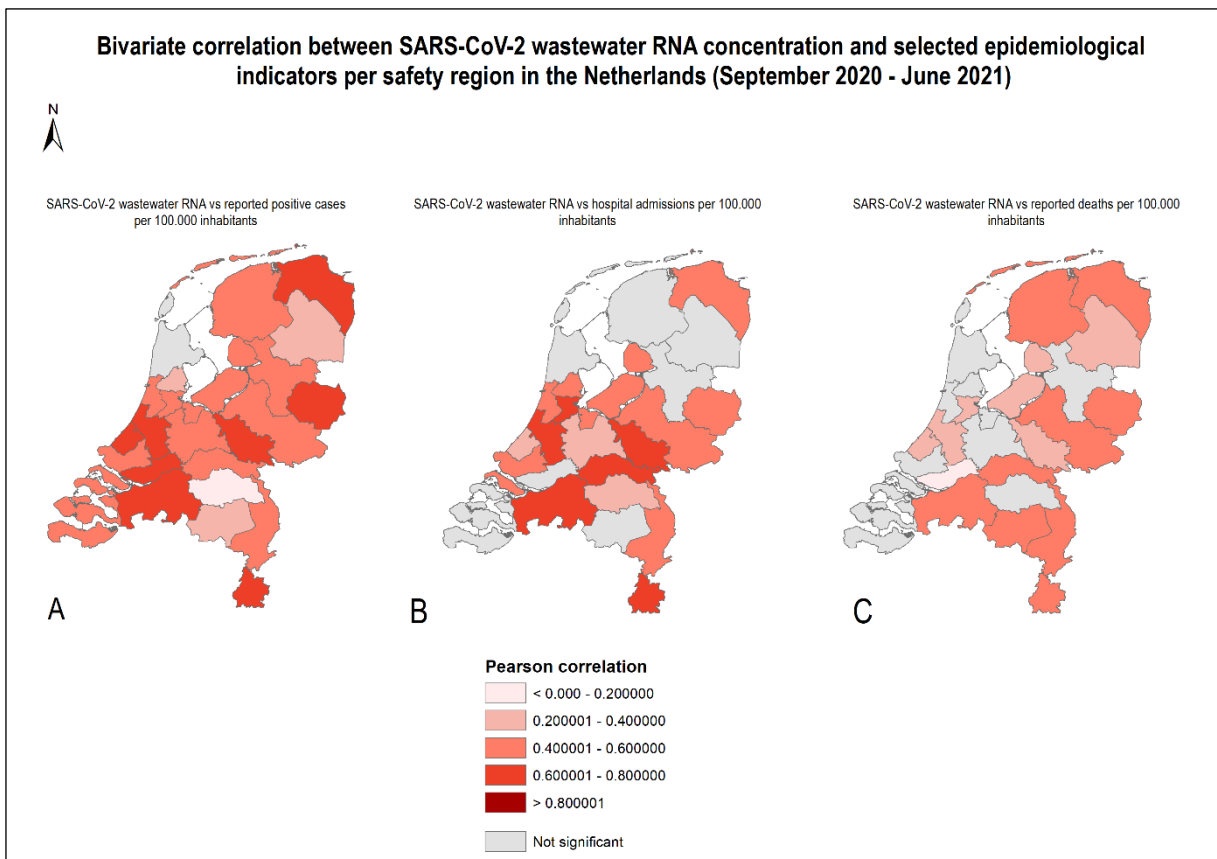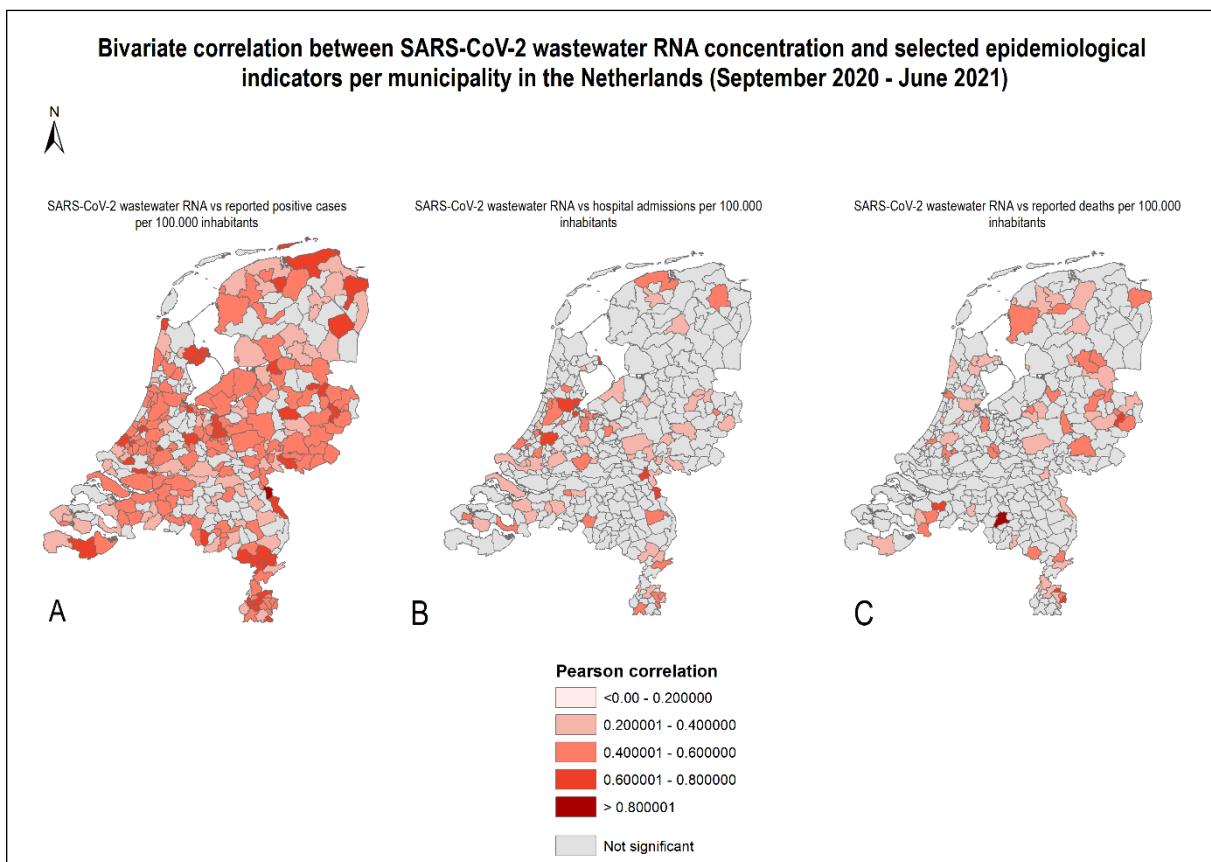

Figure 4-7: Correlation coefficients per municipality between SARS-CoV-2 wastewater RNA concentration and (A) reported positive cases, (B) hospital admissions and (C) reported deaths per 100.000 inhabitant in the Netherlands from September 2020 to June 2021.

4.3.   Cross correlation analysis of SARS-CoV-2 wastewater RNA and reported positive cases

The concordance between the trends in normalized SARS-CoV-2 wastewater RNA concentration and positive cases was examined using the cross-correlation function (CCF) analysis to find the time shift between the trends, and the results were visualized at the municipality level. Research hypothesis, four, of this study is to identify if the SARS-CoV-2 wastewater data is an early warning indicator of the virus transmission intensity. The visual comparison of the spatio-temporal trends in the SARS-CoV-2 wastewater RNA concentration and positive cases shows the relationship between the trends but is not sufficient to describe which indicator precedes the other or if an increase in one indicator within a week is equivalent to an increase in the other within the same week. Hence the need to describe if there exists a time-shift between the SARS-CoV-2 wastewater data and the positive cases. The time shift analysis involves exploration of the trends in SARS-CoV-2 wastewater data and the positive cases to determine which indicator precedes or is synchronous with the other.

Using the cross-correlation analysis, with a statistical significance of 95% confidence interval and a maximum displacement of + or - 2 weeks, a time graph was generated showing the time shift and the CCF value between the wastewater RNA concentration and positive cases including the strongest correlation (Appendix 1). The time graph was generated for all the municipalities and the results were interpreted and recorded in a table (Appendix 2). Figure 4-8 shows that there are 300 statistically significant municipalities and 52 statistically nonsignificant municipalities based on the cross-correlation analysis. Out of the 300 statistically significant municipalities, 148 (49%) have no shift in time, which indicates that an increase or decrease in SARS-CoV-2 wastewater RNA concentration occurs at the same time period as the increase or decrease in positive cases. In 77 (26%) municipalities, the SARS-CoV-2 wastewater concentration precedes the positive cases by one week and in 70 (23%) municipalities by two weeks. However, 4 (1%) reveal that the positive cases precede the SARS-CoV-2 wastewater concentration by one week and only 1 municipality reveals this by two weeks. The spatial variation of the cross-correlation between the SARS-CoV-2 wastewater RNA and reported positive cases is presented at the municipality level (Appendix 3B). It indicates the CCF value of the highest correlation between SARS-CoV-2 wastewater data and positive cases. Appendix 3B indicates that there is a significant and varying positive correlation across 300 municipalities including most large municipalities and provides a better association between the indicators.

Overall, the SAR-CoV-2 wastewater RNA concentration precedes the positive cases in 49% (147 out of 300) of the municipalities in the Netherlands. From this result, for an increase in SARS-CoV-2 wastewater RNA concentration, there would be an increase in positive cases after one or two weeks in about half of the municipalities. Hence, the SARS-CoV-2 wastewater data could act as a suitable early warning indicator of the virus transmission intensity. A second-half, 49% (148 out of 300) of the municipalities, show a synchronous time (zero time-shift) relationship between the SARS-CoV-2 wastewater RNA concentration and the positive cases, indicating an increase in the wastewater concentration as the cases increase within the same week. The zero-time shift relationship might be an effect of the weekly collection of the wastewater data. To investigate the effect of this weekly temporal resolution, the resulting zero-time shift cross-correlation was analyzed to examine the time shift of the second-highest correlation in SARS-CoV-2 wastewater RNA concentration and positive cases. The cross-correlation graphs (Appendix 1) show a close relationship between the highest cross-correlation and the second-highest correlation, hence, the need to assess the time shift of the second highest correlation. The 49% (148 out of 300) municipalities with zero time shift (Figure 4-8B) – indicating the two indicators (SARS-CoV-2 wastewater RNA concentration and the positive cases) occur at the same time, were considered for the second-highest correlation analysis to understand if a further time shift exists between these indicators.
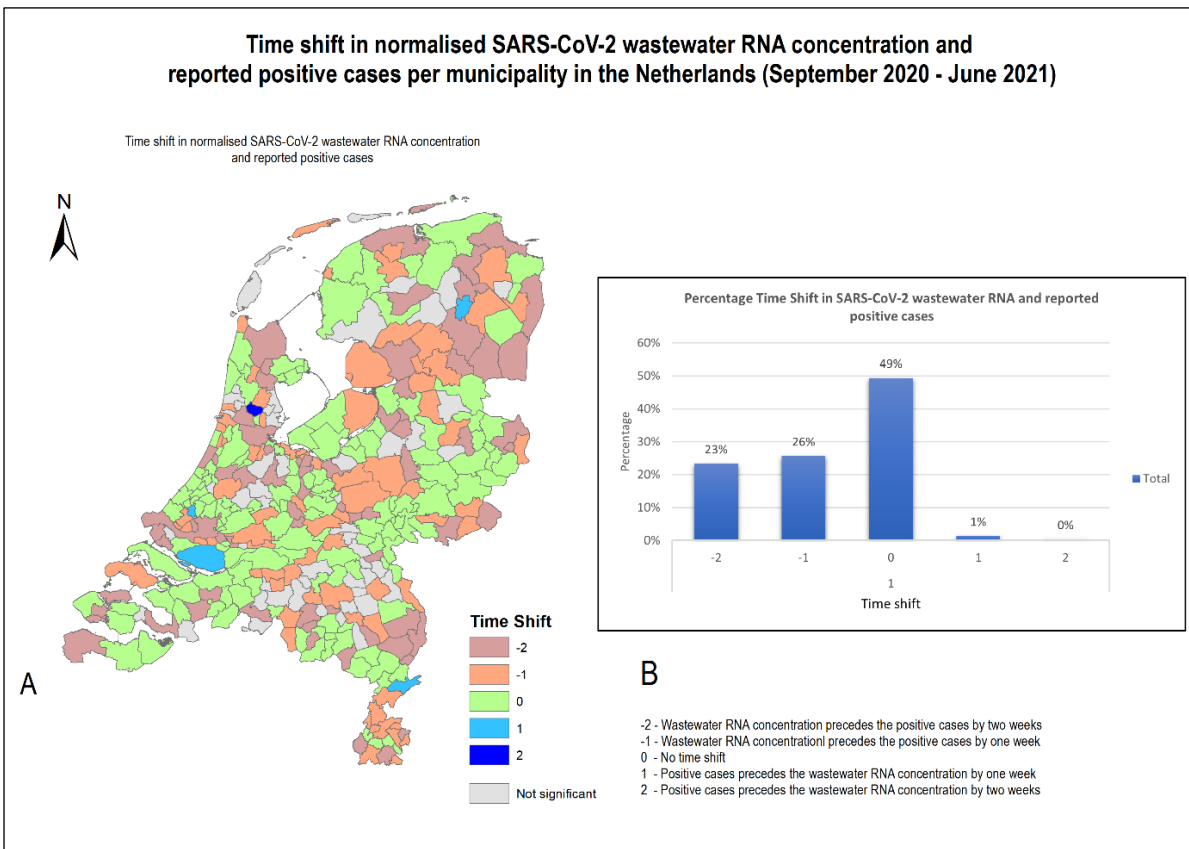
Figure 4-8: Time shift between the SARS-CoV-2 wastewater RNA concentration and the reported positive cases per 100.00 inhabitants in the Netherlands

From the assessment of the zero-time shift municipalities, the results show 37% municipalities where the SARS-CoV-2 RNA wastewater concentration precedes the positive cases by one week and 30% municipalities by two weeks. However, 34% indicate that the positive cases precede the SARS-CoV-2 wastewater RNA concentration by one week, indicating an increase in positive cases observed a week before an increase in the SARS-CoV-2 wastewater RNA concentration. In summary, the result of the time shift of the second highest correlation, a majority of the municipalities, show SARS-CoV-2 RNA wastewater concentration increase before the positive cases increase by one or two weeks. Hence, the SARS-CoV-2 wastewater data as shown to be feasible as an early warning indicator of the virus transmission intensity.
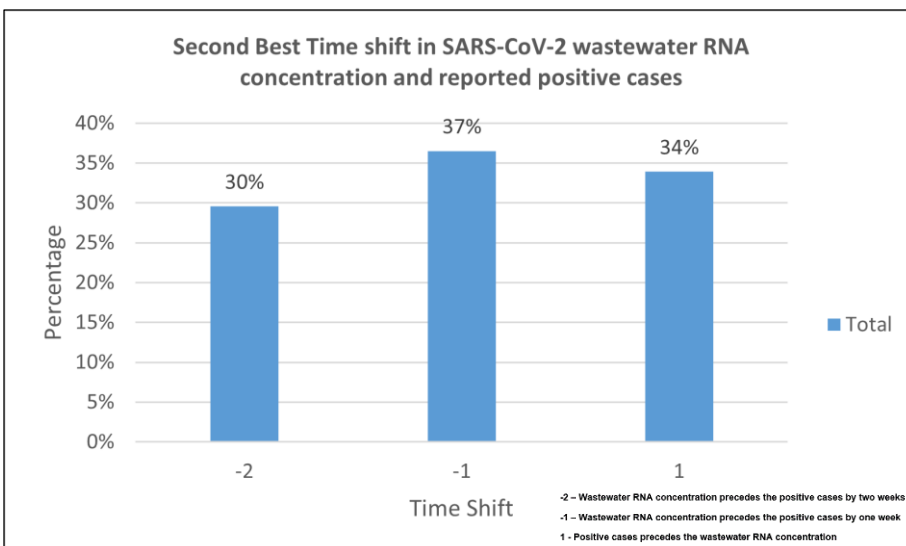

Figure 4-9: Second Time Shift in SARS-CoV-2 wastewater RNA concentration and reported positive cases

5.   DISCUSSION

This chapter discusses the results of the spatial-temporal exploration of SARS-CoV-2 wastewater RNA concentration and selected epidemiological indicators, the correlation between the wastewater RNA concentration and the selected epidemiological indicators, and elaborates on the potential of SARS-CoV-2 wastewater data as an early warning indicator for transmission of SARS-CoV-2. Besides, evidence-based recommendations are derived from the results and analysis. The chapter also highlights the limitations of the research.

5.1.   Variations in SARS-CoV-2 wastewater RNA ocncentration and SARS-CoV-2 transmission in space and time

There are weekly variations in normalised SARS-CoV-2 wastewater RNA concentration with distinct peaks and decline in the virus intensity during the study period across the national, safety region and municipality levels. The study demonstrates that the SARS-CoV-2 wastewater RNA data is suitable to describe the spatiotemporal patterns of SARS-CoV-2 transmission across multiple geographical scales. The organisation and integration of the SARS-CoV-2 epidemiological indicators datasets have allowed a combination of space and time visualisation to support this study. Unlike other studies which have analysed only the temporal variation of SARS-CoV-2 wastewater RNA from hospitals (Gonçalves et al., 2021), universities (Scott et al., 2021), or cities serving a single wastewater treatment plant (D'Aoust et al., 2021), multiple cities (Medema et al., 2020), individual municipalities (Kaplan et al., 2020; Prado et al., 2021), multiple municipalities (Saguti et al., 2021), a single region (D'Aoust et al., 2021; Kumar et al., 2021) or multiple regions (Korber et al., 2020). Our study has explored the SARS-CoV-2 wastewater RNA together with other epidemiological indicators at the national level, safety region, and municipality level. Furthermore, it highlights the usefulness of the SARS-CoV-2 wastewater data for temporal variation on a local scale monitored over time. Our analysis confirms that the SARS-CoV-2 wastewater RNA data is a useful complementary epidemiological indicator to explore the spatiotemporal variation of the SARS-CoV-2 transmission intensity.

5.2.   Associations between SARS-CoV-2 wastewater RNA concentration and other epidemiological indicators at different geographical scales

SARS-CoV-2 wastewater with positive cases, hospital admissions and reported deaths are statistically associated at different geographical scales, including national, safety regions and municipality levels. Spatial variations of the statistical association decrease as the geographical scales increase, with strongest associations at the smallest scale: the municipal level, providing a detailed picture in municipalities. This is consistent with the findings of Nemudryi et al. (2020) that geographically smaller-scale analysis of the statistical relationship between SARS-CoV-2 wastewater RNA and COVID-19 cases is useful for observing virus transmission dynamics. Previous studies have shown statistical associations of SARS-CoV-2 wastewater concentration and some epidemiological indicators at multiple communities (Weidhaas et al., 2021), a single city (D'Aoust, Graber, et al., 2021), selected cities in the Netherlands (Medema et al., 2020), and a single municipality (Wurtz et al., 2021). However, a complete representation of the Netherlands has not been carried out so far. By including all municipalities, our study closes geographical knowledge gaps, and provides a national-wide overview. The association of the wastewater RNA concentration and other epidemiological indicators provides opportunity to monitor virus transmission in the Netherlands as a whole (Sims & Kasprzyk-Hordern, 2020). Our study has shown that analyzing the wastewater data and epidemiological indicators at municipal level can be useful for monitoring of the SARS-CoV-2 transmission intensity in smaller geographical areas.

5.3.   SARS-CoV-2 wastewater data suitable as an early warning indicator to COVID-19 virus intensity

The time-shift cross-correlation analysis (Section 4.3) indicates that increases in SARS-CoV-2 wastewater RNA concentration precede increases in weekly positive cases by one or two weeks in most municipalities in the Netherlands. In other cases, the two indicators occur synchronous, meaning that both indicators increase/decrease within the same week. Further analysis of the municipalities where the two indicators do not precede each other showed that in more than half of these municipalities, increases in SARS-CoV-2 RNA wastewater concentration precede increases in positive cases, further supporting the initial result. Other studies only have reported that the SARS-CoV-2 RNA wastewater concentration precedes the reported positive cases in a single city (Agrawal et al., 2021; D'Aoust et al., 2021), selected cities in the Netherlands (Medema et al., 2020), a municipality (Nemudryi et al., 2020) and selected urban areas (Hillary et al., 2021). Our study included all municipalities in the Netherlands to demonstrate the temporal relationship between SARS-CoV-2 RNA wastewater concentration and positive cases in these municipalities during the selected time period (September 2020 to June 2021). Through the potential predictive ability of the SARS-CoV-2 wastewater data on the virus transmission, examining the temporal relationship between SARS-CoV-2 RNA wastewater concentration and positive cases has reveal additional information in making more robust inferences (Ahmed et al., 2021). This makes SARS-CoV-2 wastewater data generally suitable as a spatially disaggregate early warning indicator of the virus transmission intensity.

Our time shift analysis result (Section 4.3) also indicates that a few municipalities show positive cases increase before the SARS-CoV-2 wastewater RNA concentration by one or two weeks. This result is similar to Weidhaas et al. (2021), which also showed that a decline in positive cases precedes a decline in wastewater SARS-CoV-2 RNA. Factors such as frequency of testing, vaccination and testing policies could impact the positive cases data (Moorlag et al., 2020). Testing quality might have improved over time during the study period due to the availability of more test centres and test kits, affecting the outcome of the trends in positive cases. The number of WWTP in a municipality, the share of the population contributing to the WWTP could impact the SARS-CoV-2 wastewater data (Hillary et al., 2021). The percentage contribution of wastewater from the municipality to the WWTP was considered in this study. The distribution of the wastewater RNA concentration from WWTP into the municipalities might impact the trends in wastewater data due to multiple municipalities contributing to one WWTP. These factors might affect the result of the time shift analysis of the two indicators. However, based on our findings, SARS-CoV-2 wastewater data acts as a complementary epidemiological indicator to the monitor virus transmission intensity.

5.4.   Recommendation for descion making and policy development

We derived evidence-based recommendations for health-related decision making and policy planning from the findings of this study (Objective 3)

5.4.1.   Recommendation based on analysis to support decision making

a) Epidemiological indicators such as positive cases, hospital admissions are established indicators to monitor the transmission of SARS-CoV-2 intensity, yet there is substantial amount of asymptomatic and presymptomatic cases that are not (always) identified through clinical testing (Larsen et al., 2020). WBE offers an approach to provide a picture of the infections at a community level, but it cannot replace the individual testing indicators. In our study, the SARS-CoV-2 wastewater data is a suitable epidemiological indicator to monitor the spatio-temporal trends in the virus transmission across different geographical levels. The wastewater data is also useful for the identification of re-emerging infections and to target areas with high infection rates.

It is recommended to use spatio-temporal SARS-CoV-2 wastewater trends across different geographical levels as a timely indicator to COVID-19 virus intensity. Based on the wastewater collection structure in different countries, national wastewater-based approach can be adopted for potential nationwide monitoring of the virus transmission.

b) In our study, the wastewater RNA data has proven to be a suitable early warning indicator for the virus transmission at a smaller and finer geographical (municipal) level. This insight could help public health officials to identify areas with high infections, where the infection rate has surpassed the action threshold set by the health authority. Finally, the early indication of infection with the wastewater RNA trends could help in the assessment of the precautionary restriction by the public health authority.

It is recommended to use SARS-CoV-2 wastewater RNA concentration data for the design and implementation of targeted public health interventions.

5.4.2. Recommendation based on data collection and data quality

a) The organisation of the SARS-CoV-2 wastewater RNA data and the other epidemiological indicators datasets to a uniform unit of analysis is an important step in this study, which allowed the ease of generation of the spatio-temporal trends. The viability of the wastewater RNA data has provided a premise for the improvement and restructuring of the data to municipal level.

It is recommended to include additional municipal information in the wastewater data collection process to facilitate the generation of spatio-temporal trends to monitor the virus at the municipal scale and complement the other clinical surveillance indicators. As, highest variations occur at the municipal scale, such collection provides a detailed picture of the health measures to be implemented by the health authorities.

b) There is a potential for under-reporting of COVID-19 positive cases due to testing from different centres and bodies. This might affect the result of the temporal association between the SARS-CoV-2 wastewater RNA concentration and the positive cases. Incorporating more interoperability in the contact tracing system among public health initiatives can help improve reporting of COVID-19 cases.

It is recommended that the public health authorities should include additional measures that would improve reporting of the number of infected people, which would enrich the quality of the data.

c) The spatial and temporal analysis of the weekly reading of the SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators has described the weekly relationship between the two indicators. The potential of the wastewater RNA data to describe the weekly transmission of the virus intensity has been established. An improvement of the result would be a smaller temporal resolution to improve the results of our analysis.

It is recommended to facilitate a more frequent (e.g., daily, 2-daily) collection of wastewater samples.

5.5.   Research limitations

This study explores datasets of different epidemiological indicators from different sources. The data used are of different spatial units and needed to be organised to a uniform unit of analysis. The first limitation of that the open data is that some positive cases might have gone unreported, particularly in the earlier stages of the pandemic. Some testing organisations perform the clinical testing and might not report to the municipal health authority charged with reporting the number of people positive for the virus, thereby not fully reflecting the actual number of infections. Secondly, some missing data were observed in the datasets which might have affected the results. Another limitation is the information of the catchment area of the WWTP. As there are instances where multiple municipalities contribute to a single WWTP, the percentage municipality-to-WWTP contribution was considered. The percentage contributions might not fully represent the actual situation.

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

This thesis presents a comparative approach to assess the potential of SARS-CoV-2 wastewater data in relation to some selected epidemiological indicators; reported positive cases, hospital admissions, ICU admissions and reported deaths in the Netherlands using spatially disaggregated publicly available secondary data. A spatial and temporal approach was explored to analyze the relationship between these indicators and derive an evidence-based recommendation from the relationship.

A methodological approach was developed to combine open-source data of the selected epidemiological indicators from different sources, integrating them for analysis across different geographical scales (national, safety region and municipality). This showed how combining data at a disaggregated level to a uniform spatial unit can provide additional valuable information in understanding virus transmission in the Netherlands.

Statistical and spatial analyses were applied to describe the spatio-temporal relationship between the SARS-CoV-2 wastewater RNA concentration and the selected epidemiological indicators, and specifically SARS-CoV-2 wastewater RNA across national, safety region and municipality levels. This allowed a visual comparison of the virus transmission in space and time and showed prevailing spatial and temporal patterns in the SARS-CoV-2 wastewater RNA data, additionally providing an opportunity to observe the emergence and re-emergence of SARS-CoV-2 transmission across these three geographical scales.

The statistical relationship between SARS-CoV-2 wastewater RNA concentration and three epidemiological indicators, positive cases, hospital admissions and reported deaths has shown that a smaller scale provides the most information when analysing the wastewater data and other epidemiological indicators; hence, the municipal level showed the most robust scale in describing the SARS-CoV-2 transmission intensity. The temporal association between the SARS-CoV-2 wastewater RNA concentration and positive cases, showed an increase in SARS-CoV-2 wastewater RNA concentration preceding an increase in positive cases by one or two weeks is present in most municipalities in the Netherlands.

This thesis establishes the SARS-CoV-2 wastewater data is a complementary epidemiological indicator to monitor the spatial and temporal variation of the virus transmission across national, safety region and municipality scales in the Netherlands. It emphasizes that SARS-CoV-2 wastewater concentration data at the municipal level can support targeted monitoring. It further concludes that the SARS-CoV-2 wastewater data is suitable to act as an early warning indicator of SARS-CoV-2 transmission intensity, useful for timely public health interventions and make informed decisions.

6.2. Future research and next steps

Future research can explore a finer temporal resolution – e.g., the daily positive cases - and SARS-CoV-2 wastewater concentration, to gain new insights on SARS-CoV-2 wastewater data as a complementary indicator to the standard epidemiological indicators. This approach could be extended to consider factors determining the viral load of coronavirus in wastewater. Factors that determine the number of infections, like vaccinations and testing, could be considered as well. Besides, it could be interesting to see whether similar approaches in this study can be done in other countries.

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APPENDIX

Appendix 1 - Cross correlation analysis Graph
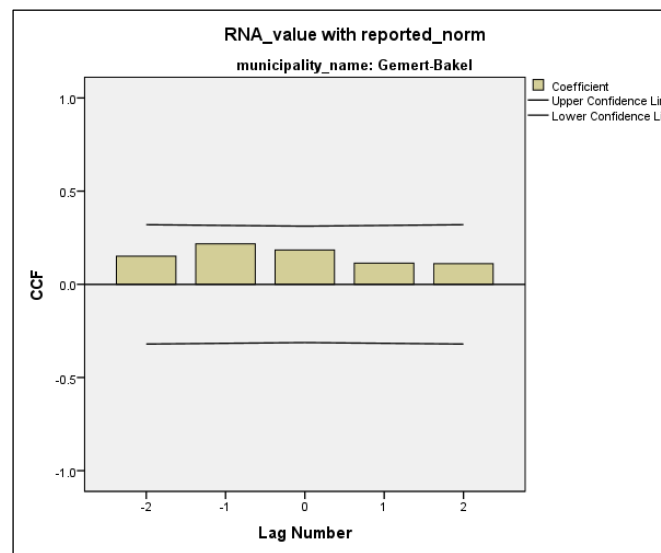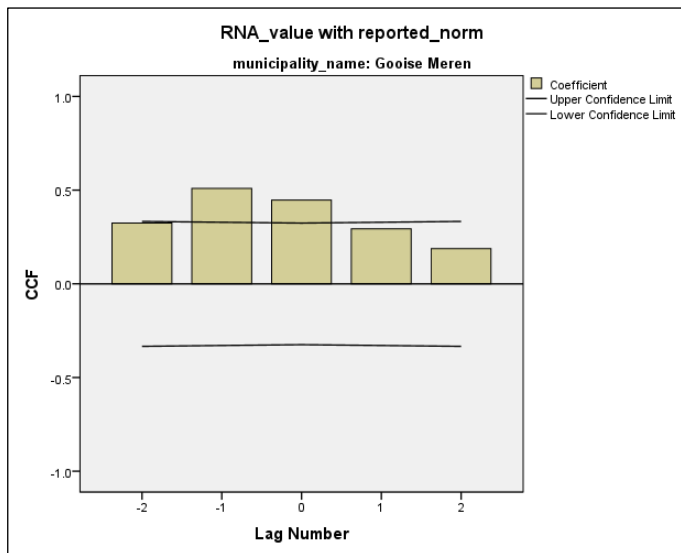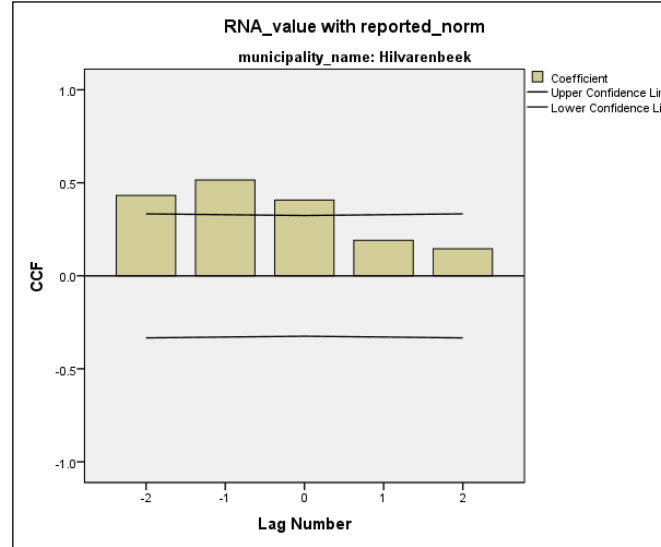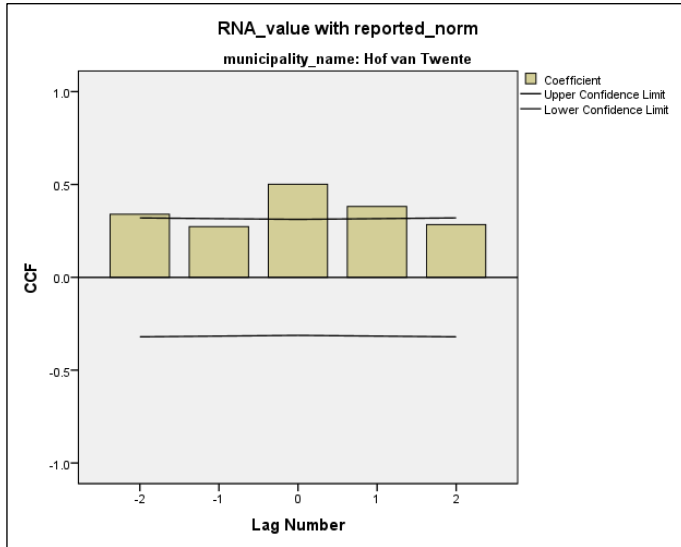
Appendix 2 - Table showing some examples of the cross-correlation analysis results, the time graph interpretation for the best and second-best time shift, the highest correlation and the second highest correlation

S/N	Municipality name	Municipality code	Cross correlation	Best Time shift	Significance	Second highest correlation	Significance	Second Best Time shift
1	Groningen	GM0014	0.321	-2	1	0.305	1	0
2	Almere	GM0034	0.455	0	1	0.451	1	-1
3	Stadskanaal	GM0037	0.307	-1	1	0.304	1	0
4	Veendam	GM0047	0.279	-2	0	0.207	0	-1
5	Zeewolde	GM0050	0.567	0	1	0.426	1	1
6	Achtkarspelen	GM0059	0.632	0	1	0.467	1	-2
7	Ameland	GM0060	0.233	0	0	0.22	0	-2
8	Harlingen	GM0072	0.498	-1	1	0.473	1	0
9	Heerenveen	GM0074	0.436	0	1	0.403	1	-2
10	Leeuwarden	GM0080	0.418	0	1	0.402	1	-1
11	Ooststellingwerf	GM0085	0.247	-2	0	0.225	0	-1
12	Opsterland	GM0086	0.338	-2	1	0.222	0	-1
13	Schiermonnikoog	GM0088	0.701	-2	1	0.632	1	0
14	Smallingerland	GM0090	0.294	-2	0	0.256	0	0
15	Terschelling	GM0093	0.412	-1	1	0.231	0	0
16	Vlieland	GM0096	0.244	-2	0	0.113	0	-1
17	Weststellingwerf	GM0098	0.318	-1	1	0.277	0	-2
18	Assen	GM0106	0.342	1	1	0.317	1	-1
19	Coevorden	GM0109	0.466	-2	1	0.42	1	-1
20	Emmen	GM0114	0.401	-2	1	0.368	1	-1
21	Hoogeveen	GM0118	0.416	-2	1	0.385	1	0
22	Meppel	GM0119	0.31	-1	1	0.282	0	0
23	Almelo	GM0141	0.577	0	1	0.522	1	-1
24	Borne	GM0147	0.602	0	1	0.523	1	1
25	Dalfsen	GM0148	0.312	-1	1	0.261	0	0
26	Deventer	GM0150	0.643	0	1	0.613	1	-1
27	Enschede	GM0153	0.598	0	1	0.512	1	-2
28	Haaksbergen	GM0158	0.48	0	1	0.384	1	1
29	Hardenberg	GM0160	0.558	0	1	0.429	1	-1
30	Hellendoorn	GM0163	0.474	-1	1	0.452	1	0
31	Hengelo (O.)	GM0164	0.666	0	1	0.585	1	1
32	Kampen	GM0166	0.342	0	1	0.154	0	1
33	Losser	GM0168	0.483	-1	1	0.428	1	-2
34	Noordoostpolder	GM0171	0.513	-1	1	0.431	1	-2

Appendix 3 - Bivariate correlation coefficients and cross correlation coefficients per municipality between SARS-CoV-2 wastewater RNA concentration and reported positive cases per 100.000 inhabitants in the Netherlands.

Bivariate correlation and Cross correlation of SARS-CoV-2 wastewater RNA concentration and reported positive cases per municipality in the Netherlands (September 2020 - June 2021)